THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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2024 EDQM virtual training programme:

Independent modules on European Pharmacopoeia texts related to Biologicals and on Microbiology chapters

(Live Webinars)
Date: 30 January 2024 – 01 February 2024

Module 1
General Concepts, Biotherapeutics and ATMPs

30 January 2024

Module 1 Agenda

Ph. Eur. general concepts

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Biotherapeutics

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Advanced therapy medicinal products (ATMPs)

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Module 1:
General concepts

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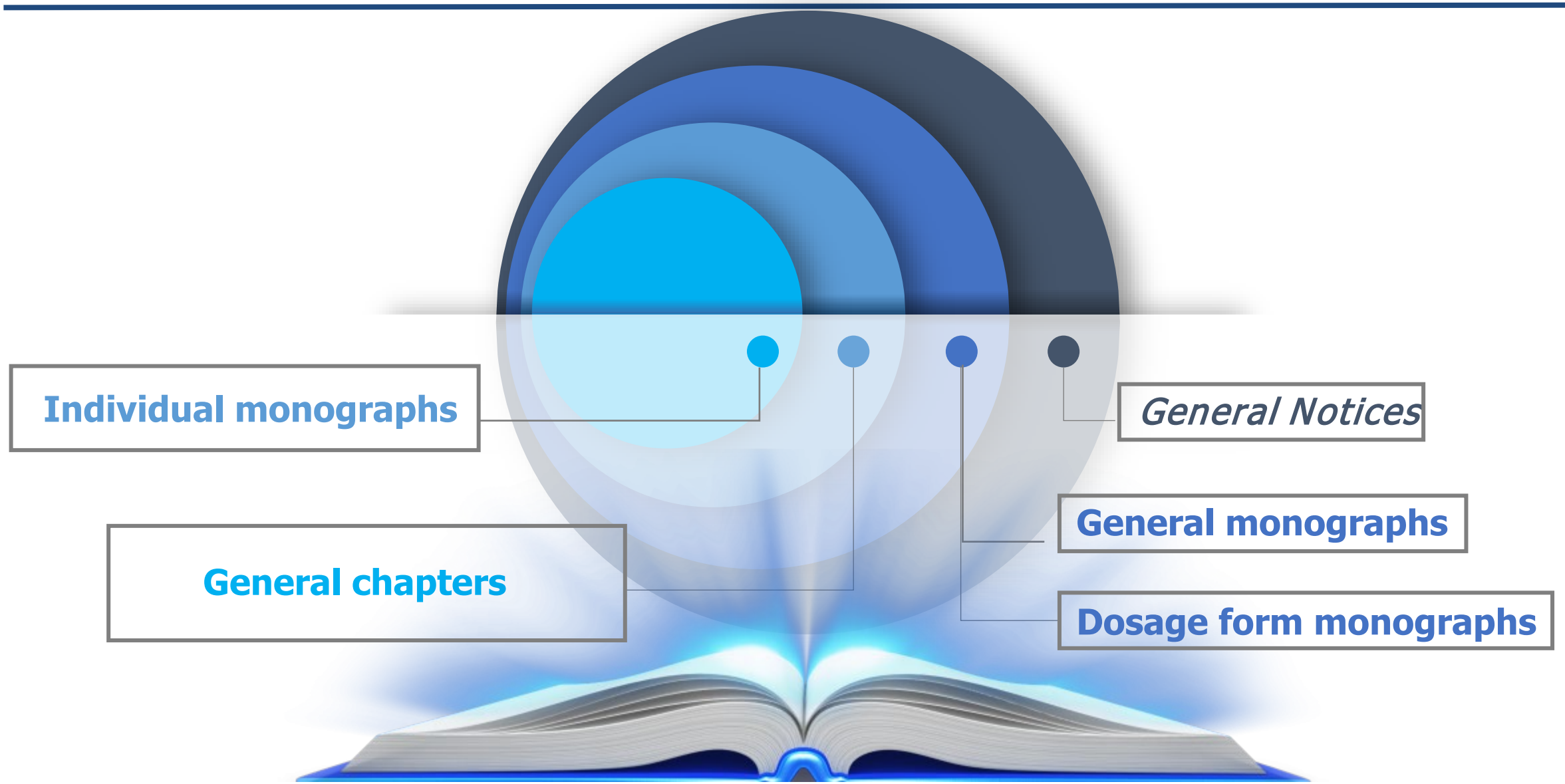
(Live Webinar)
Date: 30 January 2024

Outline

- Structure of the Ph. Eur. & general principles
  - *General Notices*
  - General monographs – dosage form monographs
  - General chapters
  - Individual monographs
- An overview of general chapters 5.26 and 5.27

Ph. Eur.: Content and structure



General Notices

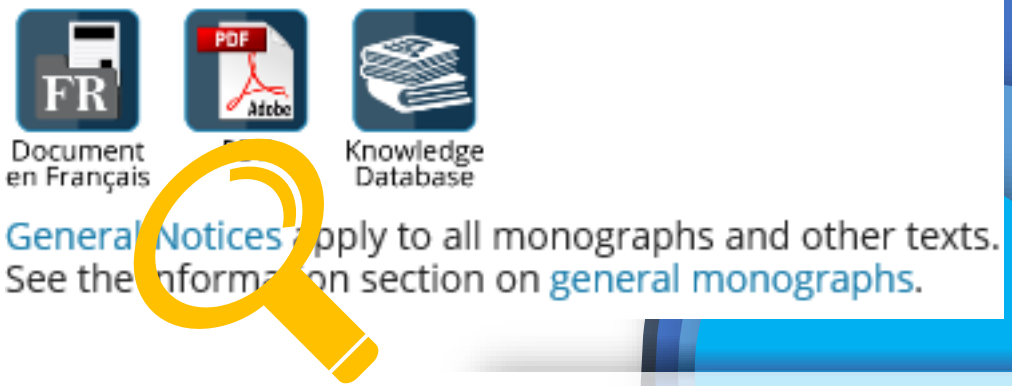
General Notices – answers to a lot of questions!

- Such as:
  - What does *compliance* mean?
  - What is mandatory, what is not?
  - What to do when *implementing* a pharmacopoeial procedure?
  - What about *alternative* analytical procedures?
  - What about *waiving* of tests?
  - Human or veterinary use?
  - What does "suitable" mean?

And many more…

An on-demand webinar is available if you want to learn more about the recent changes
https://www.edqm.eu/en/-/getting-the-big-picture-what-has-changed-in-the-ph.-eur.-general-notices

General Notices



General Notices

General Notices apply to all monographs and other texts.
See the information section on general monographs.

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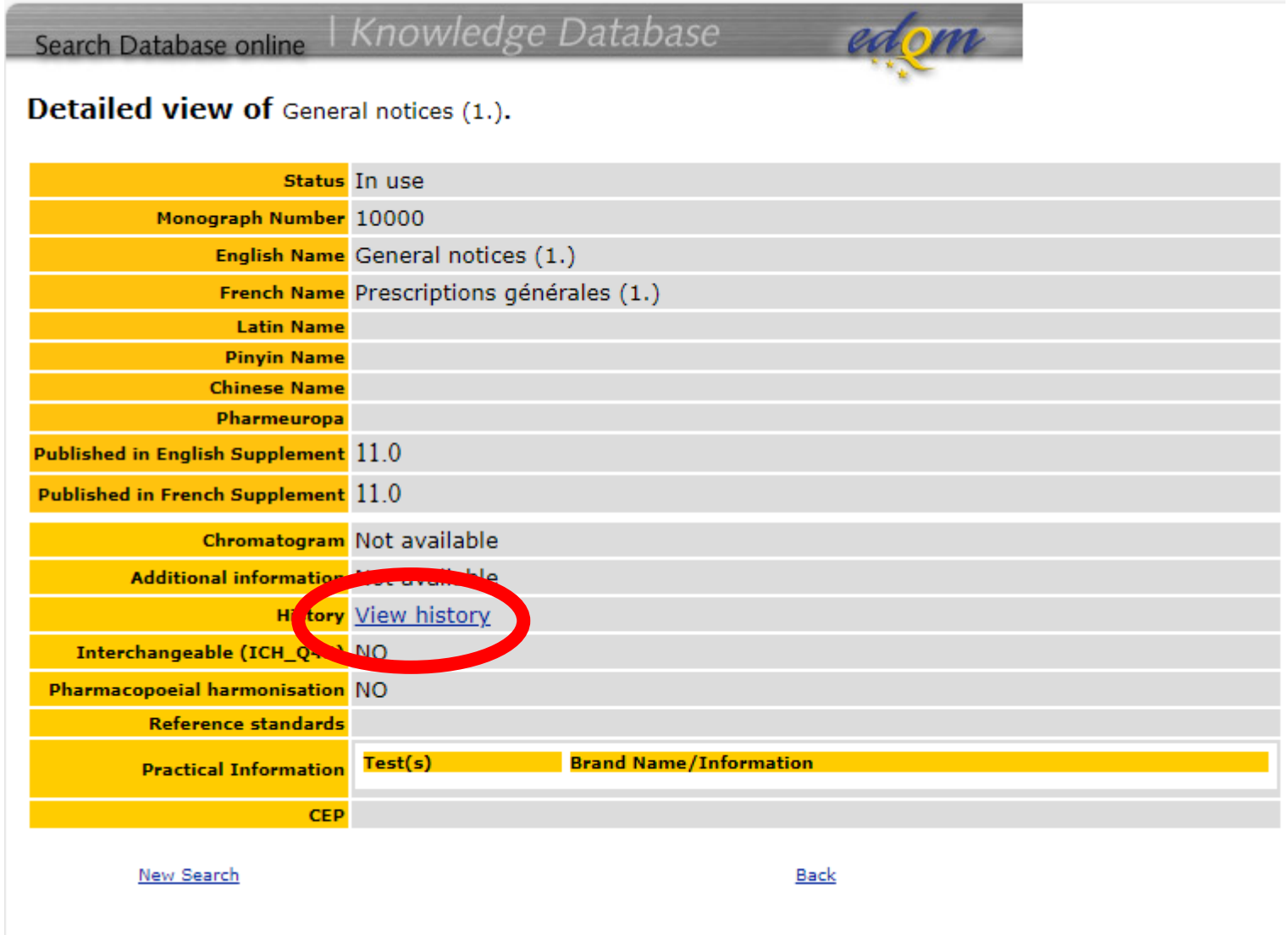
1. GENERAL NOTICES



SUPPLEMENT 10.7

The General Notices (Ph. Eur. chapter 1) have been revised to clarify a number of items for users, to review their structure and contents and to include a section on medicinal products containing chemically defined active substance
The main changes are:

- inclusion of a table of contents;
- numbering of the sections and sub-sections;
- replacement of 'method' or 'test method' by 'analytical procedure' in line with ICH Q2(R1);
- terminology harmonised and clarified to avoid multiple terms for the same thing (finished product, pharmaceutical preparation, medicinal product); 'medicinal product' used systematically;
- terms 'period of validity' and 'period of use' replaced by 'shelf life' and 're-test period', which are the terms used in the ICH guidelines (see 1.1.2.1 Scope);
- Inclusion of a sub-section on Demonstration of suitability of monographs (1.1.2.3);
- Validation and implementation of Ph. Eur. analytical procedures (1.1.2.4): clarification that validation of an analytical procedure provided in a general chapter that is not referred to in a monograph is the responsibility of the user;
- Quantities (1.2.1): reference to new chapter 2.1.7. Balances for analytical purposes added and wording modified to fit with this chapter;
- Solutions (1.2.6): definitions added for 'freshly prepared solution' and 'immediately before use';
- General chapters (1.3): clarification of their legal status;
- Materials for containers and containers (1.3.1): more details given, in line with the recent restructuring of these chapters;
- Characters (1.5.1.7): reference to general chapter 5.11 added and the table on solubility removed; inclusion of the paragraph on polymorphism currently in II. Introduction to the Ph. Eur.;
- Identification (1.5.1.8): clarification of first and second identification series and alternative identifications;
- Tests and assays (1.5.1.9): Limits: clarification of rounding; Chiral substances: paragraph from II. Introduction to the Ph. Eur. included here; Equivalents: example given;
- Functionality-related characteristics of excipients (1.5.1.13): section originally published in the General Notices replaced by paragraph from II. Introduction to the Ph. Eur. as the wording was considered to be clearer;
- Monographs on herbal drugs (1.5.2): current paragraphs gathered under this new sub-section;
- Monographs on medicinal products containing chemically defined active substances (1.5.3): new sub-section added; paragraphs on related substances and impurities adapted from the Technical Guide for the elaboration of monog
defined active substances (Edition 2020).

Validation and implementation of Ph. Eur. analytical procedu

1.1.2.4

Analytical procedure given in an individual monograph: no need for revalidation (unless otherwise mentioned in the monograph, for example)

The analytical procedures given in an individual monograph have been validated in accordance with accepted scientific practice and recommendations on analytical validation. Unless otherwise stated in the individual monograph or in the corresponding general chapter, validation of these procedures by the user is not required.

The analytical procedures provided in general chapters may be used for active substances, excipients, medicinal products and other articles that are not covered by an individual monograph. In such cases, validation of the procedures is the responsibility of the user.

Analytical procedure provided in a general chapter: validation under the responsibility of the user

When implementing a Ph. Eur. analytical procedure, the user must assess whether and to what extent its suitability under the actual conditions of use needs to be demonstrated according to relevant monographs, general chapters and quality systems.

MORE DETAILS on IMPLEMENTATION IN THE NEW CHAPTER 5.26 (PH. EUR. 11th EDITION)

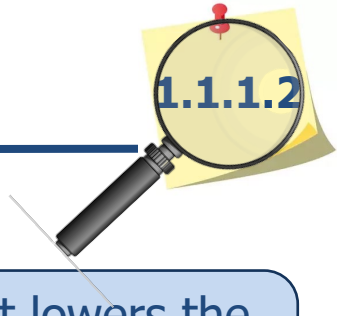
Conventional terms: meanings

'competent authority'. The national, supranational or international body / organisation vested with the authority for **making decisions concerning the issue in question**. May be a national pharmacopoeia authority (NPA), a licensing authority or an official medicines control laboratory (OMCL).

'unless otherwise justified and authorised'. Means that the requirements must be met, **unless the competent authority authorises a modification** (e.g. of an analytical procedure or limit) or an **exemption** where **justified** by the manufacturer in a particular case.

'suitable', 'appropriate'. In certain texts, the terms 'suitable' and 'appropriate' are used to describe a reagent, test, micro-organism, etc.; in such cases, if criteria for suitability are not described in the text, suitability is demonstrated to the satisfaction of the competent authority.

Scope



- The *scope of a monograph* is stated in the DEFINITION section

CALCITONIN(SALMON) 0471 Polypeptide having the structure determined for salmon calcitonin I. It lowers the calcium concentration in plasma of mammals by diminishing the rate of bone resorption. It is obtained by **chemical synthesis** or **by a method based on recombinant DNA (rDNA)** technology.

- *Human/veterinary use*
  - Unless otherwise stated, monographs cover **human and veterinary** use.
  - Where a substance is used in both human and veterinary products, the same quality specification is applied.
  - When the monograph title states "for veterinary use" the substance is intended for veterinary products only e.g. *Tetanus vaccine for veterinary use.*

- *Reference to regulatory documents*

References are provided to users of the Ph. Eur. for information. Inclusion of such a reference does not modify the status of the documents referred to, unless explicitly stated in the text.

SLIDO

What section(s) of a monograph are mandatory?

- ✓Definition
- ✓Characters
- ✓Production
- ✓Identification
- ✓Tests and assay
- ✓Storage
- ✓Labelling

Correct answers in green!

Demonstration of compliance with the Ph. Eur.

"*Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements.*"

Compliance

= compliance with all **mandatory** parts of a **monograph**

MANDATORY	INFORMATIVE
Definition Production Identification Tests Assay Labelling	Characters Storage *Functionality-related characteristics*

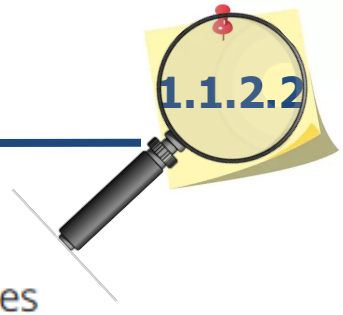
SLIDO

To demonstrate compliance, do I have to perform all the mandatory tests given in a monograph?

- ✓ Yes
- ✓ No
- ✓ I don’t know

Correct answer in green!

The way(s) to compliance - Flexibility



(1) An article is of Ph. Eur. quality if it complies with all of the requirements stated in the monograph. This does not im cribed in a monograph when assessing compl may obtain assurance that an article is of Ph. Eu ol strategy and data derived, for example, from validation studies of the manufacturing process.

In certain monographs, the sentence '*The following procedure is given as an example*' means that the analytical procedure described has been validated and may be implemented as is or may be replaced by a suitable, validated procedure (without having to demonstrate its equivalence to the '*example*' procedure), subject to approval by the competent authority.

(2) An enhanced approach to quality control could utilise process analytical technology (PAT) and/or real-time rele eal-time luded by t

(3) Reduction of animal testing: the Ph. Eur. is committed to phasing out the use of animals for test purposes, in accordance with the 3Rs (Replacement, Reduction, Refinement) set out in the European Convention for the Pr Scientific Purposes. In demonstrating compl y consider establishing additional syste the competent authority, the choice of tests performed to assess compliance with the Ph. Eur. when animal tests are prescribed is established in such a way that animal usage is kept to a minimum.

(1) WAIVING OF TESTS

(2) PROCESS ANALYTICAL TECHNOLOGY

(3) SUPPORTING THE 3Rs

Alternative analytical procedures

"The tests and assays described are the official analytical procedures upon which the standards of the Ph. Eur. are based. With the agreement of the competent authority, alternative analytical procedures may be used for control purposes, provided that they enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official procedures were used. In the event of doubt or dispute, the analytical procedures of the Ph. Eur. are alone authoritative."

- ✓ Users' responsibility to demonstrate comparability to the satisfaction of the *competent authority*
- ✓ Compliance required, but alternative procedures may be used: same pass/fail decision
- ✓ The pharmacopoeial procedure remains the reference procedure

NEW CHAPTER 5.27
COMPARABILITY OF ALTERNATIVE ANALYTICAL PROCEDURES

General monographs

Dosage form monographs

General monographs & Dosage form monographs

General monographs

- Classes of substances/medicinal products
- Mandatory for all substances/products within scope of their definition
- Aspects that cannot be included in each individual monograph
- Not cross-referenced in individual monographs (exceptions)

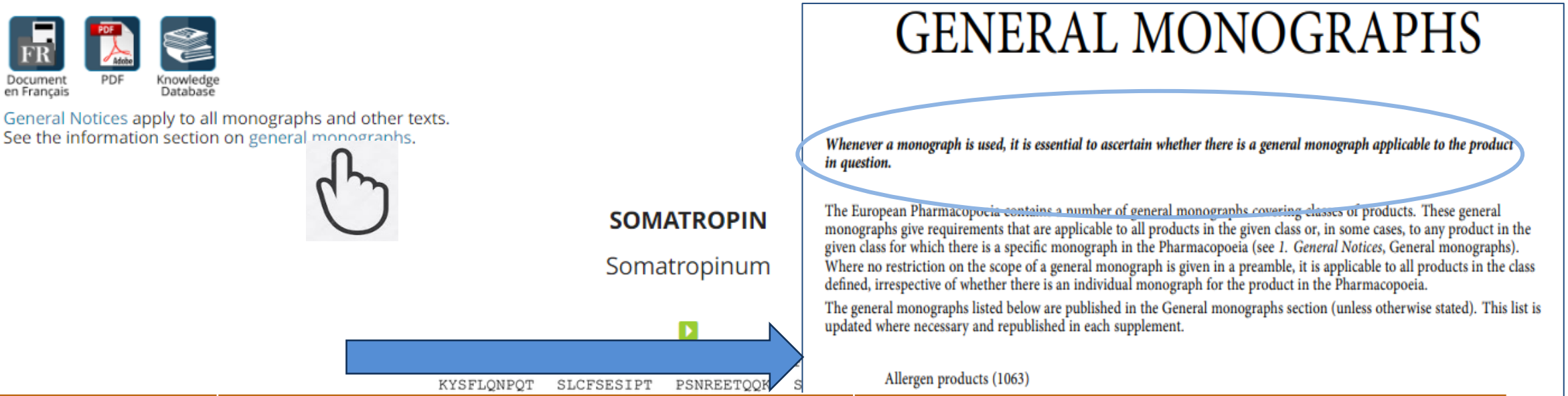
- Ex.: *Products of recombinant DNA technology* (0784), *Allergen products* (1063), *Vaccines for vet. use* (0062)

Dosage form monographs

- Mandatory for all medicinal products within scope of their definition

Ex.: *Parenteral preparations* (0520)

General monographs: Somatropin injection case study



	API	Medicinal product
Individual monographs	*Somatropin* (0951)	*Somatropin injection* (2370)
General monographs	*Substances for pharmaceutical use* (2034) + *Products of rDNA technology* (0784)	*Pharmaceutical preparations* (2619) + *Products of rDNA technology* (0784) + *Products with risk of transmitting agents of animal spongiform encephalopathies* (1483)
Dosage form monographs		*Parenteral preparations* (0520)

List of general monographs

Allergen products (1063)

Chemical precursors for radiopharmaceutical preparations (2902)

Dosage Forms

(published in the Dosage forms section or the Homoeopathic preparations section, as appropriate)

Essential oils (2098)

Herbal drug extracts (0765)

Herbal drug preparations (1434)

Herbal drugs (1433)

Herbal drugs for homoeopathic preparations (2045)

(published in the Homoeopathic preparations section)

Herbal teas (1435)

Herbal teas, instant (2620)

Homoeopathic preparations (1038)

(published in the Homoeopathic preparations section)

Immunosera for human use, animal (0084)

Immunosera for veterinary use (0030)

Live biotherapeutic products for human use (3053)

Methods of preparation of homoeopathic stocks and potentisation (2371)

(published in the Homoeopathic preparations section)

Monoclonal antibodies for human use (2031)

Mother tinctures for homoeopathic preparations (2029)

(published in the Homoeopathic Preparations section)

Pharmaceutical preparations (2619)

Products of fermentation (1468)

Products with risk of transmitting agents of animal spongiform encephalopathies (1483)

Radiopharmaceutical preparations (0125)

Recombinant DNA technology, products of (0784)

Substances for pharmaceutical use (2034)

Vaccines for human use (0153)

Vaccines for veterinary use (0062)

Vegetable fatty oils (1579)

Addressed in this presentation

Addressed in other modules of this training session

Not addressed in this training session

Substances for pharmaceutical use (2034)

- Definition: *Substances for pharmaceutical use are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use.*
- Requirements laid down in this general monograph apply to all substances for pharmaceutical use whether or not the substance is covered by an individual monograph.
- Consists of the following sections: production, characters, identification, tests, assay, labelling.
- **Biological substances fall into the scope of this monograph!**

General monograph 2034: “View History” section of KD

SUPPLEMENT 11.3
Production: addition of a paragraph explaining the Ph. Eur. approach for *N*-nitrosamines impurities. This approach has been defined based on the feedback from Heads of Medicines Agencies & European Medicines Agency groups (Joint CHMP/CVMP Quality Working Party, Biologics Working Party, Committee for Veterinary Medicinal Products, Herbal Medicinal Products Committee, Homeopathic Medicinal Products Working Group) as well as from National Competent Authorities of non-EU Ph. Eur. member states.
Additional information of interest: CHMP* opinion pursuant to Article 5(3) of Regulation (EC) No 726/2004 regarding the detection, management and prevention of presence of *N*-nitrosamines in medicinal products for human use (see assessment report published on 25 June 2020**) and CHMP* decision to apply these recommendations to ''sartans medicinal products'' (see news published on 13 November 2020***).
*CHMP: Committee for Human Medicinal Products of the European Medicines Agency
**https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf (available here)
***https://www.ema.europa.eu/en/news/nitrosamines-ema-aligns-recommendations-sartans-those-other-medicines (available here)

SUPPLEMENT 10.3
Identification: to cover the various approaches used in different countries, a sentence has been added to clarify the status of the tests described under the second identification subsection of individual monographs.

SUPPLEMENT 9.3
The ICH Q3D guideline represents a change of paradigm in the control of elemental impurities by defining permitted daily exposures for elemental impurities to be applied to medicinal products. As part of the Ph. Eur. implementation strategy, references to the heavy metals tests (*2.4.8*) have been deleted from individual monographs on substances for pharmaceutical use (except those for veterinary use only). Further implementation steps include a revision of this general monograph to clarify the expectations with regard to elemental impurities for substances for pharmaceutical use.
Production. Section updated accordingly, as only the manufacturer of a substance for pharmaceutical use knows which elemental impurities may be potentially introduced as catalysts and reagents, and whose levels would therefore need to be controlled. Control in this context should be understood as a comprehensive approach following the principles of risk management, which may include analytical testing if appropriate.
Elemental impurities. A new subsection has been added to explain the absence of tests in monographs on substances for pharmaceutical use unless otherwise prescribed.

SUPPLEMENT 9.1
Related substances. The reference to the EMA guideline on the limits of genotoxic impurities has been replaced by a reference to the new ICH guideline M7 *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*.
Bacterial endotoxins. The requirements associated with the test have been clarified and aligned with the European Pharmacopoeia **policy** on bacterial endotoxins in substances for pharmaceutical use (see Pharmeuropa - Technical information - version September 2014, revised February 2015). This revision goes hand-in-hand with the revision of general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*, published in Supplement 8.8, which includes recommendations for establishing limits and information on how to evaluate the pyrogenicity of substances.

SUPPLEMENT 8.8
The general monograph *Substances for pharmaceutical use (2034)* has been revised to exclude chemical precursors for radiopharmaceutical preparations from its scope following the creation of the general monograph *Chemical precursors for radiopharmaceutical preparations (2902)*, which is published in the same supplement.

EDITION 8.0: corrected

SUPPLEMENT 7.7
Following the elaboration of general methods for the determination of methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid (*2.5.37*) and in active substances (*2.5.38*), the Production and Related substances sections have been updated in consultation with the Joint CHMP/CVMP Quality Working Party and the CHMP Safety Working Party.

SUPPLEMENT 7.5: corrected

SUPPLEMENT 6.5
Identification: this section has been modified in accordance with the revised General Notices.
Related substances: the general monograph has been revised in line with the conclusions of the EDQM symposium 'New impurities control: setting specifications for antibiotics and synthetic peptides' held in September 2006; general provisions for the reporting, identification and qualification thresholds applicable to synthetic peptides have been added, and general chapter *5.10. Control of impurities in substances for pharmaceutical use* has been modified accordingly; in addition, the thresholds for active substances for veterinary use only have been updated according to the recently revised VICH guideline on impurities in new veterinary drug substances (EMEA/CVMP/VICH/837/99- Rev.1).
Labelling: 'added substance' has been replaced by the term 'excipient', as defined in the revised General Notices published in Supplement 6.5; the last sentence has been deleted since it is covered by the last indent.

SUPPLEMENT 6.3
Definition: the scope of the monograph was previously limited to substances for which there is an individual monograph in the Pharmacopoeia; this revision extends the scope to all substances for pharmaceutical use, but allows for the possibility of an exception for substances used in the manufacture of medicinal products for the special needs of individual patients, where this is justified by risk assessment; exceptions are also introduced for homoeopathic products.
Production: a paragraph has been added indicating that the processing of active substances with excipients is considered to be a pharmaceutical manufacturing operation that must be carried out in GMP conditions.

EDITION 6.0
Related substances: the phrase "where justified and authorised" has been added for the application of the contents of table 2034.-1.
Microbiological quality: precisions have been added to clarify the application of Table 5.1.4.-2. - Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use contained in the recently revised chapter *5.1.4. Microbiological quality of pharmaceutical preparations*.
Labelling: where appropriate, the label states the concentration of any added substance.

SUPPLEMENT 5.8
Definition: addition of a reference to new chapter *5.1.7. Viral safety*.

SUPPLEMENT 5.7
Definition: addition of a statement that the monograph does not apply to herbal drugs, herbal drug preparations or extracts. This revision stems from a request for clarification from the Herbal Medicinal Products Committee of the EMEA. The recently adopted revision of the general chapter *5.1.4. Microbiological quality of pharmaceutical preparations* has general acceptance criteria for the category ‘substances for pharmaceutical use’. Since the chapter also has provisions for herbal drugs and herbal drug preparations, it has been decided to indicate that they are not within the category ‘substances for pharmaceutical use’.

SUPPLEMENT 5.5
In the section dealing with related substances, the possibility of exemptions to the general provisions has been introduced, since it is now seen to be appropriate to make exceptions in some specific monographs.
The section on residual solvents has been modified to state explicitly that the content of residual solvents is taken into account for calculation of specific optical rotation and specific absorbance.

Substances for pharmaceutical use (2034)

Residual solvents
Chapter 5.4 (ICH Q3C)

Microbiological quality

Table 5.1.4.-2. – *Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use*

	TAMC (CFU/g or CFU/mL)	TYMC (CFU/g or CFU/mL)
Substances for pharmaceutical use	10^3	10^2

Elemental impurities
Chapter 5.20 (ICH Q3D)

Related substance
Reporting, identification and qualificati thresholds, Chapter 5.10 (ICH Q3A)

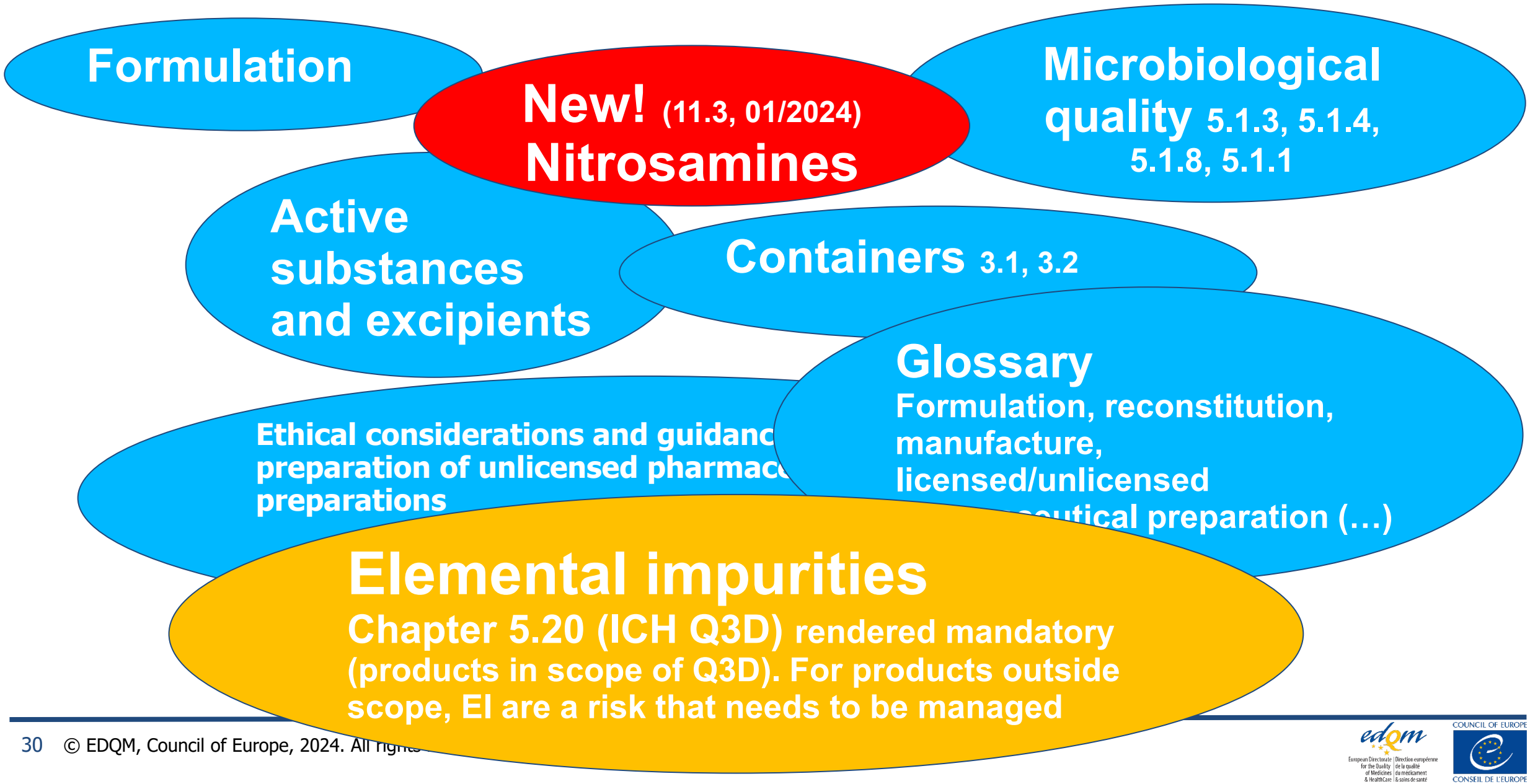
New! (11.3, 01/2024)
Nitrosamines
Chapter 2.5.42
N-Nitrosamines in active substances

Pharmaceutical preparations (2619)

INTRODUCTION

- Not a guide on how to manufacture as there is specific guidance available covering methods of manufacture and associated controls.
- Does not cover investigational medicinal products, but competent authorities may refer to pharmacopoeial standards when authorising clinical trials using investigational medicinal products.
- Applies to preparations for human and veterinary use

Pharmaceutical preparations (2619)



Products of rDNA technology (0784)

View History of KD SUPPLEMENT 9.7 (2019)

- The monograph has undergone a general revision to take into account current practices and advances in the field of recombinant DNA technology.
- **Scope**: the scope has been clarified and extended to include modified proteins, proteins obtained in transgenic animals and plants, and recombinant vaccine antigens.
- **Production**: the section has been entirely re-structured and modernised in line with the requirements of ICH, EMA and WHO guidelines for recombinant proteins. A subsection on the characterisation of the active substance has been introduced, and outlines the elucidation of recombinant protein properties including structure determination, content, biological activity, purity profile, analysis of any post-translational modifications (e.g. glycosylation) and of any other intentional modification. Likewise, a subsection briefly describing the establishment of a control strategy and how release specifications fit into the overall strategy has also been introduced.
- **Identification, Tests, Assay**: general considerations regarding the identification and assay of recombinant products, and for testing at the active substance and finished product stages, have been introduced.

SLIDO

- **I am producing a recombinant vaccine that has no individual monograph. Is it covered by any other Ph. Eur. text?**

✓No, it is not covered by a Ph. Eur. text: recombinant vaccines are excluded from the general monograph on *Products of rDNA technology* (0784)

✓Yes, it is covered by the general monograph on *Products of rDNA technology* (0784)

✓Yes, it is covered by the general monograph on *Vaccines for Human use* (0153)

✓Yes, it is covered by the general monograph on *Pharmaceutical preparations* (2619)

Correct answers in green!

Products of rDNA technology (0784)

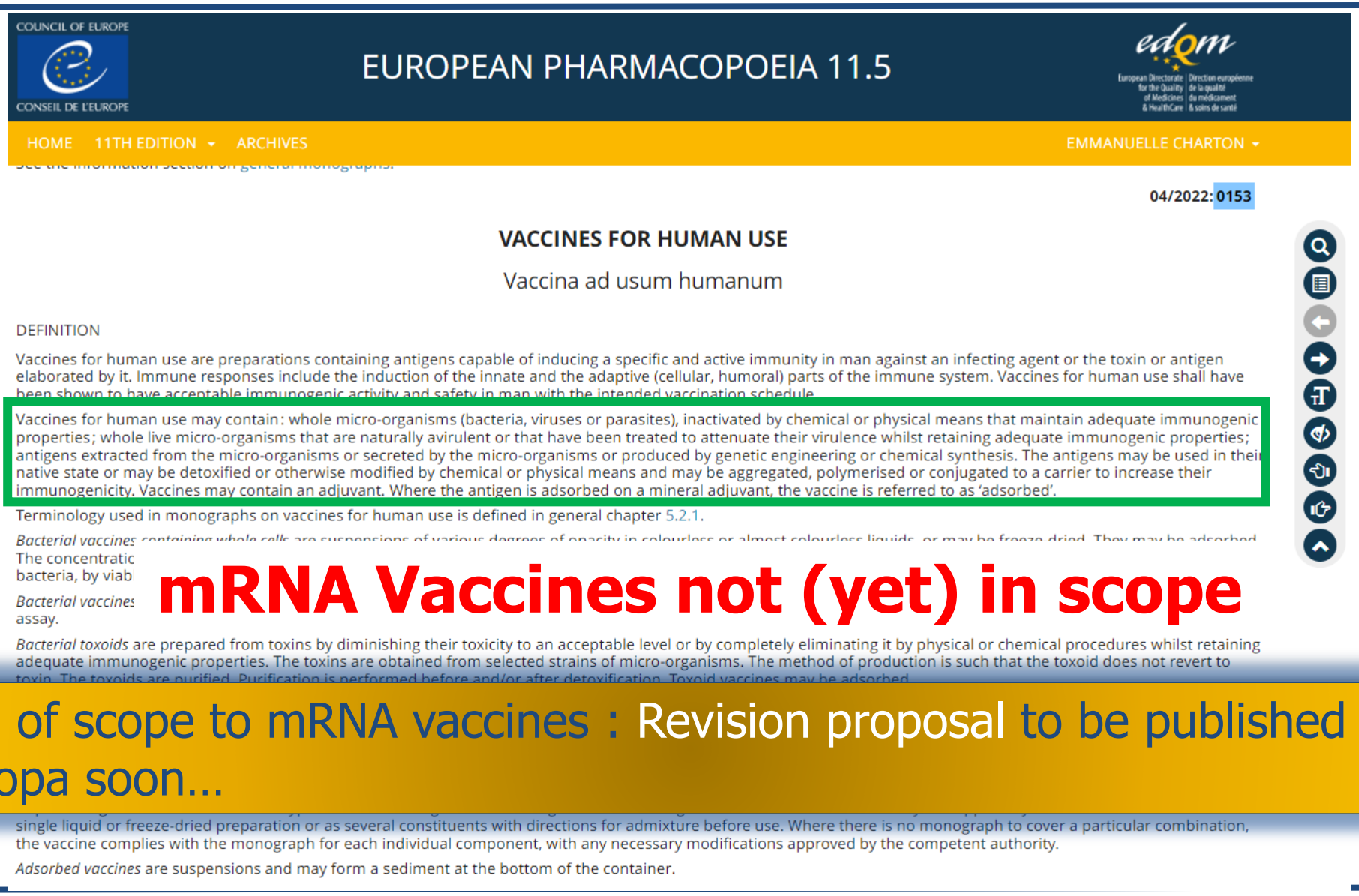
Reference to the general monograph on Vaccines for veterinary use (0062) to be included in a future revision

SLIDO

- **I am producing an mRNA vaccine (human/veterinary) that has no individual monograph. Is it covered by any other Ph. Eur. text?**
  - ✓No, it is not covered by a Ph. Eur. text
  - ✓Yes, it is covered by the general monograph on *Products of rDNA technology* (0784)
  - ✓Yes, it is covered by the general monograph on *Vaccines for human use* (0153)
  - ✓Yes, it is covered by the general monograph on *Vaccines for veterinary use* (0062)
  - ✓Yes, it is covered by the general monograph on *Pharmaceutical preparations* (2619)

Correct answer in green!

Vaccines for human use (0153)



SCOPE

mRNA Vaccines not (yet) in scope

Expansion of scope to mRNA vaccines : Revision proposal to be published in Pharmeuropa soon…

Vaccines for veterinary use (0062)

SCOPE

Expansion of scope to DNA and RNA vaccines : Revision proposal in Pharmeuropa 36.2

mRNA Vaccines not (yet) in scope

Dosage form monographs

Parenteral preparations (0520)

Parenteral preparations EUROPEAN PHARMACOPOEIA 11.0

07/2021:0520

PARENTERAL PREPARATIONS

Parenteralia

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations or to radiopharmaceutical preparations. Special requirements may apply to preparations for veterinary use depending on the animal species for which the preparation is

DEFINITION

Parenteral preparations are sterile preparations intended for administration into the human or animal body. They may be administered by injection, infusion or implantation.

They are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. Liquid preparations for injection or infusion are solutions, colloidal dispersions, emulsions or suspensions.

Parenteral preparations may contain suitable excipients, for example to adjust the tonicity of the preparation relative to blood, to adjust or stabilise the pH, to increase the solubility of the active substances, to stabilise the preparation or to provide adequate antimicrobial properties. The excipients do not adversely affect the intended medicinal action or, at the concentrations used, cause toxicity or undue local irritation.

Wherever possible, containers for parenteral preparations are made from materials that are sufficiently transparent to permit the visual inspection of the contents, except for implants and in other justified and authorised cases.

Where applicable, containers for parenteral preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections). Parenteral preparations intended for chronic use or total parenteral nutrition should have appropriate limits for specific components or elements, taking long-term toxicity into account.

Parenteral preparations are supplied in glass containers (*3.2.1*) or in other containers such as plastic containers (*3.2.2*, *3.2.2.1* and *3.2.9*) and prefilled syringes. The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent micro-organisms and other contaminants from entering the container and closure system and usually permit the withdrawal of a part or all of the contents without the closure being removed. The plastic materials or elastomers (*3.2.9*) used to manufacture the closures are sufficiently firm and elastic to allow the passage of a needle without shedding of particles contaminating the preparation. Closures for multidose containers are sufficiently elastic to ensure that the puncture is resealed when the needle is withdrawn.

Several categories of parenteral preparations may be distinguished:

- injections;
- infusions;
- concentrates for injections or infusions;
- powders for injections or infusions;
- gels for injection;
- implants;
- intravitreal preparations.

PRODUCTION

During the development of parenteral preparations whose formulation contains a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

Parenteral preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

Water used in the manufacture of parenteral preparations complies with the requirements for water for injections in bulk given in the monograph *Water for injections (0169).*

Liquid preparations for injection or infusion, examined under suitable conditions of visibility, are practically free from particles.

Recommendations on testing for visible particles are given in general chapter 5.17.2.

TESTS

Particulate contamination: sub-visible particles (*2.9.19*). Liquid preparations for injection or infusion, if applicable after reconstitution, comply with the test. Unless otherwise justified and authorised, suspensions, emulsions and gels for injection comply with the test.

In the case of preparations for subcutaneous or intramuscular injection, higher limits may be appropriate. Radiopharmaceutical preparations are exempt from these requirements. Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filtrate complies with the test.

For preparations for veterinary use, when supplied in containers with a nominal content of more than 100 mL and when the content is equivalent to a dose of more than 1.4 mL per kilogram of body mass, liquid preparations for injection or infusion, if applicable after reconstitution, comply with the test for particulate contamination: sub-visible particles.

Particulate contamination: visible particles (*2.9.20*). Liquid preparations for injection or infusion, if applicable after reconstitution, examined under suitable conditions of visibility, are practically free from visible particles.

Recommendations on testing for visible particles are given in general chapter 5.17.2.

Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filtrate complies with the test.

Sterility (*2.6.1*). Parenteral preparations comply with the test.

Bacterial endotoxins - pyrogens. Parenteral preparations for human use, if applicable after reconstitution or dilution, comply with the test for bacterial endotoxins (*2.6.14*) or, where justified and authorised, with the test for pyrogens (*2.6.8*). Recommendations on the limits for bacterial endotoxins are given in general chapter *5.1.10.* The limit for intravitreal preparations is expressed per eye.

Where the label states that the preparation is free from bacterial endotoxins or that it is apyrogenic, the preparation complies with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*), respectively.

Parenteral preparations for veterinary use comply with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*) when the volume to be injected in a single dose is 15 mL or more and is equivalent to a dose of 0.2 mL or more per kilogram of body mass.

STORAGE

In a sterile, airtight, tamper-evident container.

988 *See the information section on general monographs (cover pages)*

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations or to radiopharmaceutical preparations.

Flexibility is brought for specific cases (including biologicals) for which a quality attribute might not be applicable

"Ultimately, the implementation process runs under the user's responsibility and its successful outcome needs to be demonstrated and documented to the satisfaction of the competent authority." Chapter 5.26 Implementation of pharmacopoeial procedures (5.26.)

Parenteral preparations (0520) Major revision in Supplement 10.5 2021

Parenteral preparations EUROPEAN PHARMACOPOEIA 11.0

07/2021:0520

PARENTERAL PREPARATIONS

Parenteralia

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations or to radiopharmaceutical preparations. Special requirements may apply to preparations for veterinary use depending on the animal species for which the preparation is intended.

DEFINITION

Parenteral preparations are sterile preparations intended for administration into the human or animal body. They may be administered by injection, infusion or implantation.

They are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. Liquid preparations for injection or infusion are solutions, colloidal dispersions, emulsions or suspensions.

Parenteral preparations may contain suitable excipients, for example to adjust the tonicity of the preparation relative to blood, to adjust or stabilise the pH, to increase the solubility of the active substances, to stabilise the preparation or to provide adequate antimicrobial properties. The excipients do not adversely affect the intended medicinal action or, at the concentrations used, cause toxicity or undue local irritation.

Wherever possible, containers for parenteral preparations are made from materials that are sufficiently transparent to permit the visual inspection of the contents, except for implants and in other justified and authorised cases.

Where applicable, containers for parenteral preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections). Parenteral preparations intended for chronic use or total parenteral nutrition should have appropriate limits for specific components or elements, taking long-term toxicity into account.

Parenteral preparations are supplied in glass containers (*3.2.1*) or in other containers such as plastic containers (*3.2.2*, *3.2.2.1* and *3.2.9*) and prefilled syringes. The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent micro-organisms and other contaminants from entering the container and closure system and usually permit the withdrawal of a part or all of the contents without the closure being removed. The plastic materials or elastomers (*3.2.9*) used to manufacture the closures are sufficiently firm and elastic to allow the passage of a needle without shedding of particles contaminating the preparation. Closures for multidose containers are sufficiently elastic to ensure that the puncture is resealed when the needle is withdrawn.

Several categories of parenteral preparations may be distinguished:

- injections;
- infusions;
- concentrates for injections or infusions;
- powders for injections or infusions;
- gels for injection;
- implants;
- intravitreal preparations.

PRODUCTION

parenteral preparations whose formulation contains a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

Parenteral preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

Water used in the manufacture of parenteral preparations complies with the requirements for water for injections in bulk given in the monograph Water for injections (0169).

Liquid preparations for injection or infusion, examined under suitable conditions of visibility, are practically free from particles.

Recommendations on testing for visible particles are given in general chapter 5.17.2.

TESTS

Particulate contamination: sub-visible particles (*2.9.19*). Liquid preparations for injection or infusion, if applicable after reconstitution, comply with the test. Unless otherwise justified and authorised, suspensions, emulsions and gels for injection comply with the test.

In the case of preparations for subcutaneous or intramuscular injection, higher limits may be appropriate. Radiopharmaceutical preparations are exempt from these requirements. Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filtrate complies with the test.

For preparations for veterinary use, when supplied in containers with a nominal content of more than 100 mL and when the content is equivalent to a dose of more than 1.4 mL per kilogram of body mass, liquid preparations for injection test for particulate contamination: sub-visible particles.

Particulate contamination: visible particles (*2.9.20*). Liquid preparations for injection or infusion, if applicable after reconstitution, examined under suitable conditions of visibility, are practically free from visible particles.

Recommendations on testing for visible particles are given in general chapter 5.17.2.

Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filtrate complies with the test.

Sterility (*2.6.1*). Parenteral preparations comply with the test.

Bacterial endotoxins - pyrogens. Parenteral preparations for human use, if applicable after reconstitution or dilution, comply with the test for bacterial endotoxins (*2.6.14*) or, where justified and authorised, with the test for pyrogens (*2.6.8*). Recommendations on the limits for bacterial endotoxins are given in general chapter *5.1.10*. The limit for intravitreal preparations is expressed per eye.

Where the label states that the preparation is free from bacterial endotoxins or that it is apyrogenic, the preparation complies with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*), respectively.

Parenteral preparations for veterinary use comply with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*) when the volume to be injected in a single dose is 15 mL or more and is equivalent to a dose of 0.2 mL or more per kilogram of body mass.

STORAGE

In a sterile, airtight, tamper-evident container.

988 *See the information section on general monographs (cover pages)*

Liquid preparations for injection or infusion, examined under suitable conditions of visibility, are practically free from particles. Recommendations on testing for visible particles are given in general chapter 5.17.2

Particulate contamination: visible particles (2.9.20). Liquid preparations for injection or infusion, if applicable after reconstitution, examined under suitable conditions of visibility, are practically free from visible particles. Recommendations on testing for visible particles are given in general chapter 5.17.2.

5.17.2 *Recommendations on testing of particulate contamination: visible particles*

- General text – non-mandatory – published for the first time in Ph. Eur. 10.3

Document en Français | PDF | Knowledge Database

Tools

General Notices apply to all monographs and other texts.
See the information section on general monographs.

01/2021:51702

5.17.2. RECOMMENDATIONS ON TESTING OF PARTICULATE CONTAMINATION: VISIBLE PARTICLES

This general chapter is non-mandatory it provides information on visible particle testing of liquid preparations that refer to general chapter 2.9.20. Particulate contamination: visible particles in their monographs. This information represents considerations used in the field of visual inspection and in the control of visible particles in medicinal products. This chapter is not intended to elaborate on good manufacturing practice requirements, but should be read in conjunction with such requirements.

- Provides information on visible particle testing of liquid preparations
- Gives detailed considerations for requirement "practically free from visible particles"

General chapters

SLIDO

- **Are general chapters mandatory?**

✓No, they are only for information

✓Only those in sections 2 and 3 are mandatory, section 5 is just for information

✓Yes, they are part of the Ph. Eur., they are all mandatory

✓These texts become mandatory when referred to in an individual monograph

✓These texts become mandatory when referred to in a general monograph

✓These texts become mandatory when referred to in another general chapter that is referred to in a monograph

Correct answers in green!

General Notices 1.3. General chapters

Mandatory when referred to in a monograph

General chapters see sections 2, 3 and 5 of the Ph. Eur. become mandatory when referred to in a monograph, unless the wording clearly indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

When a general chapter is not referred to in any monograph or general chapter, it is given for information; this is usually indicated in the preamble to the general chapter.

General chapters also become mandatory when referred to in another general chapter that is itself referred to in a monograph, unless otherwise stated.

Mandatory when referred to in a chapter which is referred to in a monograph

General chapters

General chapters (sections 2 and 3)

- avoid repeating standard procedures or requirements in each monograph
- become mandatory when referred to in a monograph
- provide standard analytical procedures, that may be used (with validation) when NOT referred to in a monograph
- general requirements for equipment, equipment qualification or calibration
- Section 3: containers

General texts (section 5)

- Often published for information and guidance
- become mandatory when referred to in a monograph
- specific to certain topics (e.g. microbiology, chemometrics)
- reproduce principles of regulatory guidelines (e.g. 5.20. *Elemental impurities* → referred to in 2034 and 2619 introduction and scope of ICH Q3D GL)
- May provide a non-mandatory framework of requirements (e.g. 5.14 *Gene transfer medicinal products*, 5.2.12 *Raw materials for the production of ATMPs*)

Chromatographic separation techniques (2.2.46)

Revised chapter (harmonised with USP and JP), Ph. Eur. 11th Edition, January 2023

- Useful definitions (dwell volume, resolution, peak-to-valley ratio etc)

- System suitability requirements for LC and GC procedures:
  - system repeatability (assay)
  - system sensitivity (tests)
  - peak symmetry [≠ normalisation] (tests and assays)

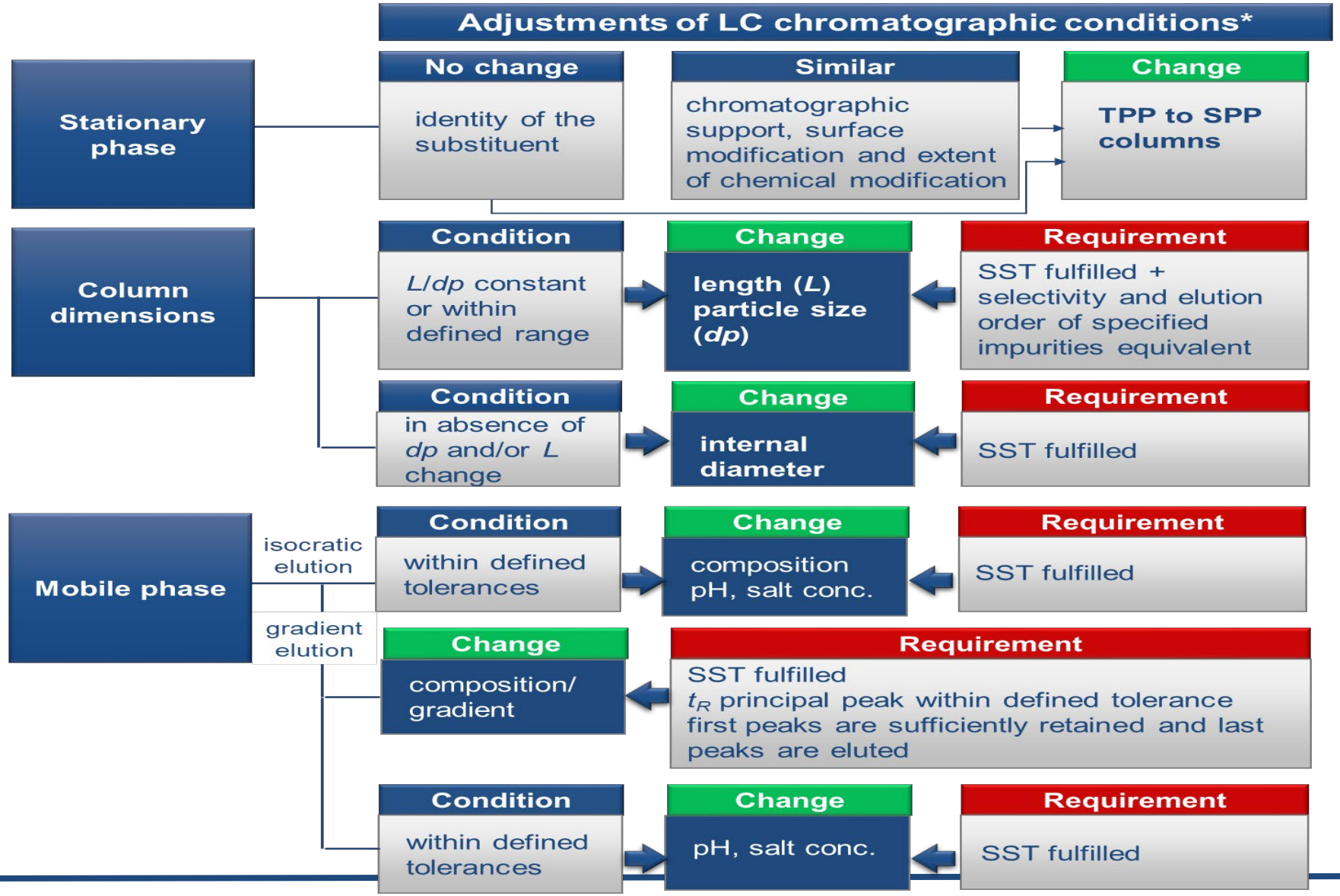
complementing those given in the individual monographs.

- Describes **framework** for **adjustment** of chromatographic conditions:
  - fulfilling the SST no longer the only trigger for adjustments
  - SST = bottom-line requirements but additional verification may be required
  - multiple adjustments

⇨ potential cumulative effects

⇨ proper evaluation / risk assessment by user

Chromatographic separation techniques (2.2.46)



*list not exhaustive (further adjustments: flow rate, injection volume)

Revised chapter (harmonised with USP and JP), Ph. Eur. 11th Edition, January 2023

Chapter 5.14. Gene therapy

Tools

General Notices apply to all monographs and other texts.
See the information section on general monographs.

04/2019:51400

5.14. GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

This general chapter is published for information.

This general chapter contains a series of texts on gene transfer medicinal products for human use. The texts provide a framework of requirements applicable to the production and

Framework of requirements – Possibility to use in the context of clinical trials
Alternatives are possible – **The competent authority decides!**

Products for Human Use (including any subsequent revisions of these documents).

Reference to EMA Guidelines

GTMP) shall mean a product obtained through a set of manufacturing processes aimed at the transfer, to be performed either *in vivo* or *ex vivo*, of a prophylactic, diagnostic or therapeutic gene (i.e. a piece of nucleic acid) to human/animal cells, and its subsequent expression *in vivo*. The gene transfer involves an expression system known as a vector, which can be of viral as well as non-viral origin. The vector can also be included in a human or animal cell.

Recombinant vectors, such as viral vectors and plasmids. Recombinant vectors are either injected directly into the patient's body (*in vivo* gene transfer) or transferred into host cells before administration of these genetically modified cells to the patient (*ex vivo* gene transfer). Viral vectors are derived from various viruses (for example, adenoviruses, poxviruses, retroviruses, lentiviruses, adeno-associated-viruses, herpesviruses). These vectors can be replicative, non-replicative or conditionally replicative. Plasmid vectors include nucleic acids in a simple formulation (for example, naked DNA) or complexed to various molecules (synthetic vectors such as lipids or polymers). Genetic material transferred by GTMPs consists of nucleotide sequences, which may notably encode gene products, antisense transcripts or ribozymes. Chemically synthesised oligonucleotides are not within the scope of this general chapter. After transfer, the genetic material may remain either cytoplasmic or episomal, or may be integrated into the host cell genome, depending on the integrating or non-integrating status of the vector.

Genetically modified cells. Genetically modified eukaryotic or bacterial cells are modified by vectors to express a product of interest.

PRODUCTION

Chapter 5.2.12 Raw materials for ATMPs

Tools

General Notices apply to all monographs and other texts.
See the information section on general monographs.

01/2017:50212
corrected 10.0

5.2.12. RAW MATERIALS OF BIOLOGICAL ORIGIN FOR THE PRODUCTION OF CELL-BASED AND GENE THERAPY MEDICINAL PRODUCTS

This general chapter is published for information.

It contains sections on the quality requirements of raw materials used for the production of cell-based and gene therapy medicinal products for human use. The provisions of the chapter do not exclude the use of different production and control methods. It is the responsibility of the manufacturer of a raw material to qualify (prove to be suitable for the intended use) the raw material in accordance with the requirements given in this general chapter.

Alternatives are possible – Responsibilities (manufacturer versus user)
Impact on the medicinal product
Risk based approach

agents (bacteria, viruses, etc.) and stability.

From a risk perspective, the use of raw materials free from human or animal substances is preferred.

The biological nature of a raw material used for the production of cell-based/gene therapy medicinal products places special requirements on its quality. Examples of the critical quality attributes specific to each class of raw material are given in this general chapter.

1. SCOPE

Chapter 5.2.8. Minimising the risk of TSE

- First published in 2001
- Identical to the EMA *Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products*
- Both texts revised in 2011 with same implementation dates

  => full alignment between Ph. Eur. and EU legislation

General Notices apply to all monographs and other texts.
See the information section on general monographs.

01/2008:1483

PRODUCTS WITH RISK OF TRANSMITTING AGENTS OF ANIMAL SPONGIFORM ENCEPHALOPATHIES

Producta cum possibili transmissione vectorium enkephalopathiarum spongiformium animalium

DEFINITION

Products with risk of transmitting agents of animal spongiform encephalopathies are those derived from tissues or secretions of animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge. This monograph applies to all substances or preparations obtained from such animals and to all substances or preparations where products obtained from such animals are included as active substances or excipients or have been used during production, for example as raw or source materials, starting materials or reagents.

PRODUCTION

Production complies with chapter *5.2.8.*

Chapter 5.2.8 is referred to in General monograph 1483 and renders chapter 5.2.8 mandatory for all substances and preparations

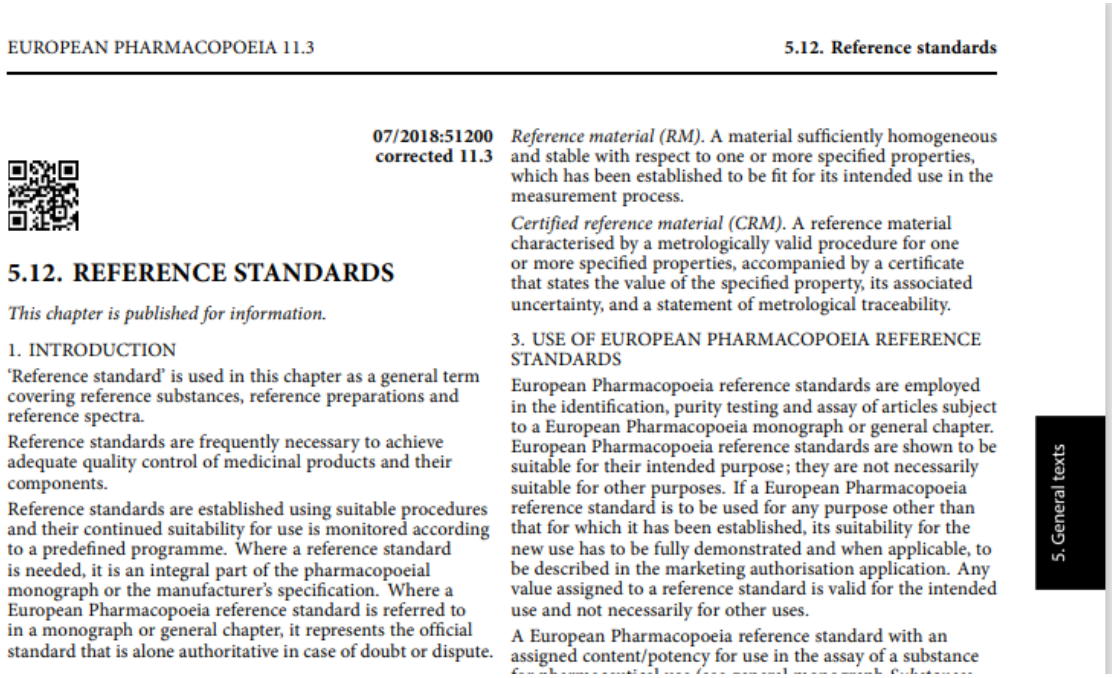
SLIDO

- **I have looked at the monograph on Trypsin: it does not contain any warnings about BSE-related issues: how can this be, knowing that the substance is of bovine origin?**

  - ✓ The monograph needs to be revised to include specific considerations on BSE/TSE
  - ✓ Trypsin presents no risk for BSE/TSE, therefore there is no indication in the monograph
  - ✓ Trypsin must comply not only with the monograph on *Trypsin* (0694) but also with the general monograph on *Products with risk of transmitting agents of animal spongiform encephalopathies* (1483), which refers to Chapter 5.2.8

Correct answer in green!

Chapter 5.12. *Reference standards*



EUROPEAN PHARMACOPOEIA 11.3 — 5.12. Reference standards

07/2018:51200
corrected 11.3

5.12. REFERENCE STANDARDS

This chapter is published for information.

1. INTRODUCTION

'Reference standard' is used in this chapter as a general term covering reference substances, reference preparations and reference spectra.

Reference standards are frequently necessary to achieve adequate quality control of medicinal products and their components.

Reference standards are established using suitable procedures and their continued suitability for use is monitored according to a predefined programme. Where a reference standard is needed, it is an integral part of the pharmacopoeial monograph or the manufacturer's specification. Where a European Pharmacopoeia reference standard is referred to in a monograph or general chapter, it represents the official standard that is alone authoritative in case of doubt or dispute.

Reference material (RM). A material sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in the measurement process.

Certified reference material (CRM). A reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that states the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

3. USE OF EUROPEAN PHARMACOPOEIA REFERENCE STANDARDS

European Pharmacopoeia reference standards are employed in the identification, purity testing and assay of articles subject to a European Pharmacopoeia monograph or general chapter. European Pharmacopoeia reference standards are shown to be suitable for their intended purpose; they are not necessarily suitable for other purposes. If a European Pharmacopoeia reference standard is to be used for any purpose other than that for which it has been established, its suitability for the new use has to be fully demonstrated and when applicable, to be described in the marketing authorisation application. Any value assigned to a reference standard is valid for the intended use and not necessarily for other uses.

A European Pharmacopoeia reference standard with an assigned content/potency for use in the assay of a substance

5. General texts

- **Terminology** (e.g. primary reference standard, secondary reference standard)
- **Recommendations** (e.g. to what extent a Ph. Eur. reference standard intended for an active substance may also be used for the corresponding pharmaceutical preparation)
- Chemical reference standards, herbal reference standards, **biological reference preparations**
- How reference standards are established
- Etc.

Referred to in general monograph 2619

Reference standards

Reference standards may be needed at various stages for quality control of pharmaceutical preparations. They are established and monitored taking due account of general chapter 5.12. Reference standards.

Individual monographs

Individual monographs

Individual monographs

- Specific but not stand-alone texts
- Analytical procedures and acceptance criteria represent required quality standards
- Based on approved specifications backed up by batch data
- Reliance on manufacturers' feedback (public consultation)

EXAMPLES

Active substances or excipients

Heparins, low molecular mass (0828)

Enoxaparin sodium (1097)

3-O-Desacyl-4'-monophosphoryl lipid A (2537)

Etanercept (2895)

Medicinal products

Insulin preparations, injectable (0854)

Human coagulation factor IX (rDNA) powder for solution for injection (2994)

Filgrastim injection (2848)

GENERAL PRINCIPLES in 1.5.1

Info on sections of individual monographs:

Production
Characters
Identification
Tests
Assay
...

SLIDO

- **Is the Production section mandatory?**

✓No, it is only for the manufacturer, to consider during their production process

✓Yes, the Production section is mandatory, as explained in the *General Notices*

✓Yes, but compliance cannot always be verified on the final article

Correct answers in green!

Production section

Cannot be verified on the final article

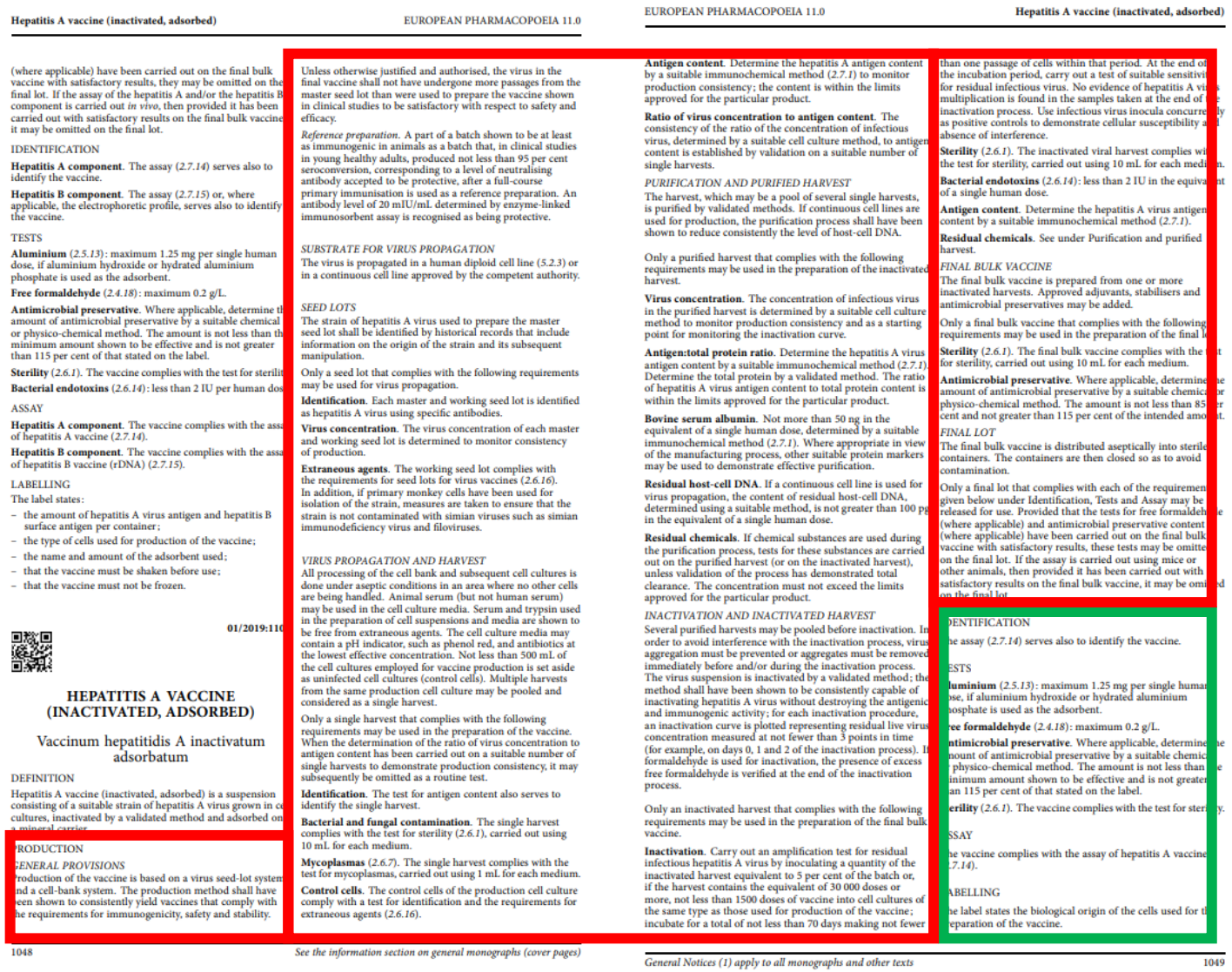
Mandatory

Statements in the Production section draw attention to particular aspects of the manufacturing process but are not necessarily exhaustive. They constitute mandatory requirements for manufacturers unless otherwise stated. They may relate, for example, to source materials, to the manufacturing process itself and its validation and control, to process-related heterogeneity of the article, to in-process testing, or to tests that are to be carried out by the manufacturer on the final article, either on selected batches or on each batch prior to release. These requirements cannot necessarily be verified on a sample of the final article by an independent analyst. The competent authority may establish that the instructions have been followed, for example, by examining data received from the manufacturer, through inspection or by testing samples.

Data evaluation - Inspections

The Production section of monographs on biologicals is a significant part of the text

Production section (vaccine monograph)



Hepatitis A vaccine (inactivated, adsorbed) EUROPEAN PHARMACOPOEIA 11.0

(where applicable) have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot. If the assay of the hepatitis A and/or the hepatitis B component is carried out *in vivo*, then provided it has been carried out with satisfactory results on the final bulk vaccine it may be omitted on the final lot.

IDENTIFICATION

Hepatitis A component. The assay (*2.7.14*) serves also to identify the vaccine.

Hepatitis B component. The assay (*2.7.15*) or, where applicable, the electrophoretic profile, serves also to identify the vaccine.

TESTS

Aluminium (*2.5.13*): maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (*2.4.18*): maximum 0.2 g/L.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility (*2.6.1*). The vaccine complies with the test for sterility

Bacterial endotoxins (*2.6.14*): less than 2 IU per human dose

ASSAY

Hepatitis A component. The vaccine complies with the assay of hepatitis A vaccine (*2.7.14*).

Hepatitis B component. The vaccine complies with the assay of hepatitis B vaccine (rDNA) (*2.7.15*).

LABELLING

The label states:

- the amount of hepatitis A virus antigen and hepatitis B surface antigen per container;
- the type of cells used for production of the vaccine;
- the name and amount of the adsorbent used;
- that the vaccine must be shaken before use;
- that the vaccine must not be frozen.

01/2019:110

HEPATITIS A VACCINE (INACTIVATED, ADSORBED)

Vaccinum hepatitidis A inactivatum adsorbatum

DEFINITION

Hepatitis A vaccine (inactivated, adsorbed) is a suspension consisting of a suitable strain of hepatitis A virus grown in cell cultures, inactivated by a validated method and adsorbed on a mineral carrier.

PRODUCTION

GENERAL PROVISIONS

Production of the vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to consistently yield vaccines that comply with the requirements for immunogenicity, safety and stability.

Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Reference preparation. A part of a batch shown to be at least as immunogenic in animals as a batch that, in clinical studies in young healthy adults, produced not less than 95 per cent seroconversion, corresponding to a level of neutralising antibody accepted to be protective, after a full-course primary immunisation is used as a reference preparation. An antibody level of 20 mIU/mL determined by enzyme-linked immunosorbent assay is recognised as being protective.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in a human diploid cell line (*5.2.3*) or in a continuous cell line approved by the competent authority.

SEED LOTS

The strain of hepatitis A virus used to prepare the master seed lot shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification. Each master and working seed lot is identified as hepatitis A virus using specific antibodies.

Virus concentration. The virus concentration of each master and working seed lot is determined to monitor consistency of production.

Extraneous agents. The working seed lot complies with the requirements for seed lots for virus vaccines (*2.6.16*). In addition, if primary monkey cells have been used for isolation of the strain, measures are taken to ensure that the strain is not contaminated with simian viruses such as simian immunodeficiency virus and filoviruses.

VIRUS PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Animal serum (but not human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator, such as phenol red, and antibiotics at the lowest effective concentration. Not less than 500 mL of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine. When the determination of the ratio of virus concentration to antigen content has been carried out on a suitable number of single harvests to demonstrate production consistency, it may subsequently be omitted as a routine test.

Identification. The test for antigen content also serves to identify the single harvest.

Bacterial and fungal contamination. The single harvest complies with the test for sterility (*2.6.1*), carried out using 10 mL for each medium.

Mycoplasmas (*2.6.7*). The single harvest complies with the test for mycoplasmas, carried out using 1 mL for each medium.

Control cells. The control cells of the production cell culture comply with a test for identification and the requirements for extraneous agents (*2.6.16*).

Antigen content. Determine the hepatitis A antigen content by a suitable immunochemical method (*2.7.1*) to monitor production consistency; the content is within the limits approved for the particular product.

Ratio of virus concentration to antigen content. The consistency of the ratio of the concentration of infectious virus, determined by a suitable cell culture method, to antigen content is established by validation on a suitable number of single harvests.

PURIFICATION AND PURIFIED HARVEST

The harvest, which may be a pool of several single harvests, is purified by validated methods. If continuous cell lines are used for production, the purification process shall have been shown to reduce consistently the level of host-cell DNA.

Only a purified harvest that complies with the following requirements may be used in the preparation of the inactivated harvest.

Virus concentration. The concentration of infectious virus in the purified harvest is determined by a suitable cell culture method to monitor production consistency and as a starting point for monitoring the inactivation curve.

Antigen:total protein ratio. Determine the hepatitis A virus antigen content by a suitable immunochemical method (*2.7.1*). Determine the total protein by a validated method. The ratio of hepatitis A virus antigen content to total protein content is within the limits approved for the particular product.

Bovine serum albumin. Not more than 50 ng in the equivalent of a single human dose, determined by a suitable immunochemical method (*2.7.1*). Where appropriate in view of the manufacturing process, other suitable protein markers may be used to demonstrate effective purification.

Residual host-cell DNA. If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method, is not greater than 100 pg in the equivalent of a single human dose.

Residual chemicals. If chemical substances are used during the purification process, tests for these substances are carried out on the purified harvest (or on the inactivated harvest), unless validation of the process has demonstrated total clearance. The concentration must not exceed the limits approved for the particular product.

INACTIVATION AND INACTIVATED HARVEST

Several purified harvests may be pooled before inactivation. In order to avoid interference with the inactivation process, virus aggregation must be prevented or aggregates must be removed immediately before and/or during the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating hepatitis A virus without destroying the antigenic and immunogenic activity; for each inactivation procedure, an inactivation curve is plotted representing residual live virus concentration measured at not fewer than 3 points in time (for example, on days 0, 1 and 2 of the inactivation process). If formaldehyde is used for inactivation, the presence of excess free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Inactivation. Carry out an amplification test for residual infectious hepatitis A virus by inoculating a quantity of the inactivated harvest equivalent to 5 per cent of the batch or, if the harvest contains the equivalent of 30 000 doses or more, not less than 1500 doses of vaccine into cell cultures of the same type as those used for production of the vaccine; incubate for a total of not less than 70 days making not fewer than one passage of cells within that period. At the end of the incubation period, carry out a test of suitable sensitivity for residual infectious virus. No evidence of hepatitis A virus multiplication is found in the samples taken at the end of the inactivation process. Use infectious virus inocula concurrently as positive controls to demonstrate cellular susceptibility and absence of interference.

Sterility (*2.6.1*). The inactivated viral harvest complies with the test for sterility, carried out using 10 mL for each medium.

Bacterial endotoxins (*2.6.14*): less than 2 IU in the equivalent of a single human dose.

Antigen content. Determine the hepatitis A virus antigen content by a suitable immunochemical method (*2.7.1*).

Residual chemicals. See under Purification and purified harvest.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated harvests. Approved adjuvants, stabilisers and antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (*2.6.1*). The final bulk vaccine complies with the test for sterility, carried out using 10 mL for each medium.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, these tests may be omitted on the final lot. If the assay is carried out using mice or other animals, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The assay (*2.7.14*) serves also to identify the vaccine.

TESTS

Aluminium (*2.5.13*): maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (*2.4.18*): maximum 0.2 g/L.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility (*2.6.1*). The vaccine complies with the test for sterility.

ASSAY

The vaccine complies with the assay of hepatitis A vaccine (*2.7.14*).

LABELLING

The label states the biological origin of the cells used for the preparation of the vaccine.

Production section

Tests on the final product

General principles - Identification section

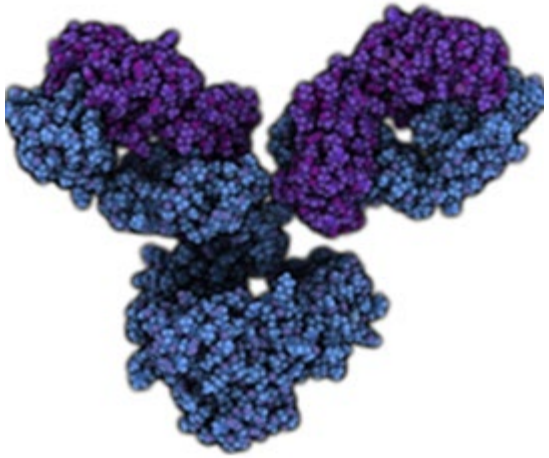
‘The tests given in the Identification section are

- not designed to give a full confirmation of the chemical structure or composition of the article;
- intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label.’

Example: mAB identification/characterisation

Quality attributes

- Molecular mass and size
- Primary structure (e.g amino acid sequence, amino acid composition
- Higher order structure (secondary and tertiary structure)
- Disulfide bonds, free thiols, cysteinylated & glutathionylated variants
- Thioether bonds
- Glycosylation (N and O-linked), glycation
- Level and type sialylation
- Amino acid modifications, substitutions
- Amino acid mis-incorporation
- Biological activity/ potency



INFLIXIMAB (2928)

Identification
A. Peptide mapping
B. Potency

General principles - tests and assays

- Scope:
  - requirements not designed to take all possible impurities into account;
  - do not presume, for example, that an impurity that is not detectable by prescribed tests is tolerated if common sense and good pharmaceutical practice require that it be absent.
- Limits:
  - take account of normal analytical errors, of acceptable variations in manufacture;
  - no further tolerances applied to prescribed limits;
  - in determining compliance: first rounded, then compared with numerical limit.

SLIDO

- **During the course of an investigation, I discover that a new analytical procedure reveals an impurity at a significant level. The methods described in the monograph do not reveal this impurity**

✓-My preparation complies with the requirements of the Ph. Eur. therefore I can release the preparation

✓My preparation complies with the requirements of the Ph. Eur. But I need to investigate this new impurity

✓I must inform the Ph. Eur. Commission/the EDQM that the monograph is insufficient

Correct answers in green!

Demonstration of suitability of monographs

Monograph insufficient?

The manufacturer must evaluate the suitability of the monograph for the quality control of their substance or medicinal product, since the choice of analytical procedures may be influenced by the manufacturing process and/or the composition of the medicinal product. In cases where the specification described in a monograph is considered to be insufficient to ensure the quality of the product or substance by a competent authority, the latter may request more-appropriate specifications from the manufacturer in line with national or regional regulations. In such cases, the competent authority informs the Ph. Eur. Commission through either the national pharmacopoeia authority or the Secretariat of the Ph. Eur. Commission (EDQM). The manufacturer is requested to provide the national pharmacopoeia authority or the EDQM with the details of the alleged insufficiency and the additional specifications applied, so that the Ph. Eur. Commission can decide on the need to revise the monograph in question.

The competent authority informs the Ph. Eur. Commission

The manufacturer provides the details

Might lead to a revision of the text

Labelling

Mandatory: statements that are necessary to demonstrate compliance

In general, labelling of medicinal products is subject to supranational and national regulation and to international agreements.

The statements in the Labelling section are not therefore comprehensive. In addition, for the purposes of the Ph. Eur., only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations.

When the term '*label*' is used in the Ph. Eur., the labelling statements may appear on the container, the package, a leaflet accompanying the package, or a certificate of analysis accompanying the article, as decided by the competent authority.

"Label": to be understood in a wide perspective (container, package, leaflet, COA, etc.)

Example: *Sucrose* monograph (0204)

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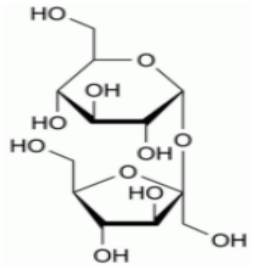
Tools

General Notices apply to all monographs and other texts.
See the information section on general monographs.

07/2024:0204

SUCROSE(1) (SEE NOTE)

Saccharum



Dextrins. If intended for use in the manufacture of large-volume parenteral preparations, it complies with the test for dextrins. To 2 mL of solution S add 8 mL of *water R*, 0.05 mL of *dilute hydrochloric acid R* and 0.05 mL of *0.05 M iodine*. The solution remains yellow.

Bacterial endotoxins (*2.6.14*): less than 0.25 IU/mg, if intended for use in the manufacture of large-volume parenteral preparations.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of large-volume parenteral preparations.

The statement in the labelling section ensures conformance to the tests for dextrins and bacterial endotoxins

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An overview of general chapters 5.26 and 5.27

Module 1: General concepts. Biotherapeutics and ATMPs

30 January 2024

Mihaela Buda, PhD
European Pharmacopoeia Department
EDQM, Council of Europe

SLIDO

1. How do I apply a pharmacopoeial procedure in my lab?

A. I use it directly to analyse my samples.

B. I first perform a full validation of the pharmacopoeial procedure.

C. I ensure the appropriate performance of the pharmacopoeial procedure in my lab.

D. I carry out verification experiments to confirm the suitability of the pharmacopoeial procedures under the actual conditions of use in my lab.

E. I do not use the pharmacopoeial procedure in my lab.

Correct answers in green!

Ph. Eur. concepts related to analytical procedures

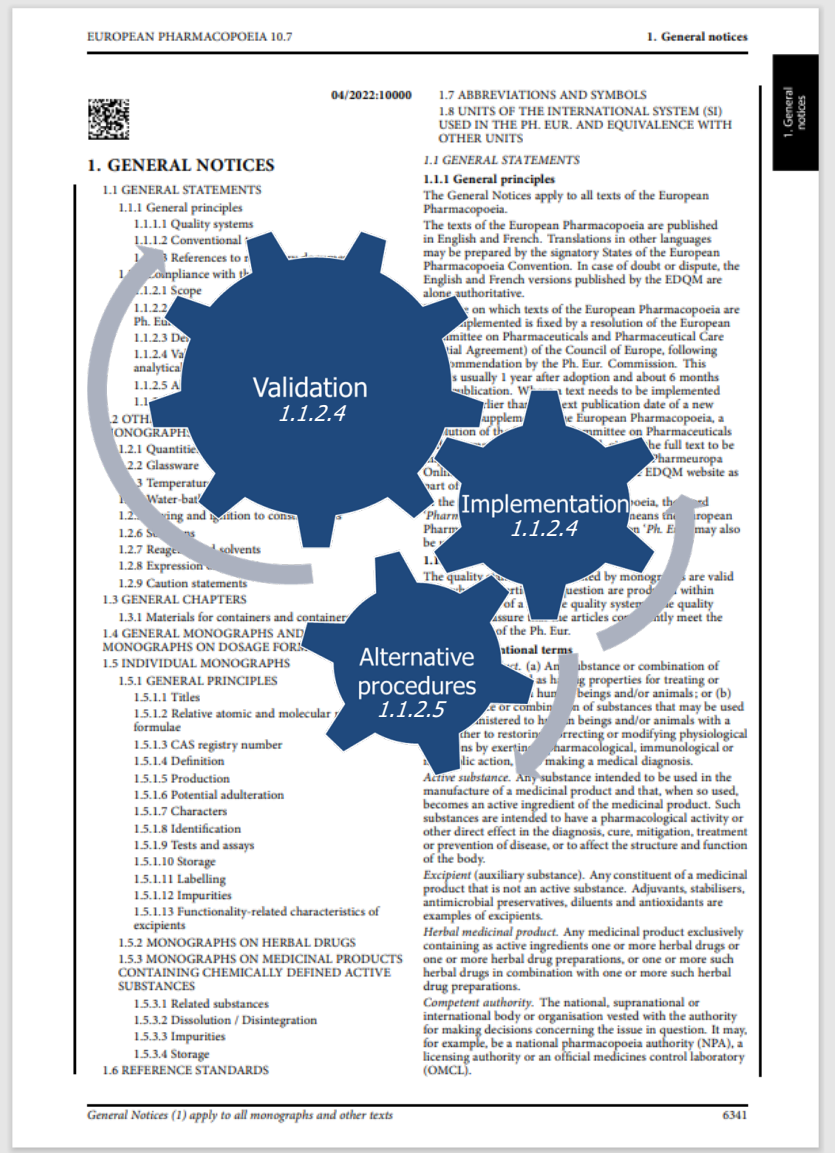
1.1.2.4 Validation and implementation of Ph. Eur. analytical procedures

The analytical procedures given in an individual monograph have been **validated** in accordance with accepted scientific practice and recommendations on analytical validation. Unless otherwise stated in the individual monograph or in the corresponding general chapter, validation of these procedures by the user is not required.

When **implementing** a Ph. Eur. analytical procedure, the user must assess whether and to what extent its suitability under the actual conditions of use needs to be demonstrated according to relevant monographs, general chapters and quality systems.

1.1.2.5 Alternative analytical procedures

The tests and assays described are the official analytical procedures upon which the **standards** of the Ph. Eur. are based. With the **agreement of the competent authority**, alternative analytical procedures may be used for control purposes, provided that they enable an **unequivocal decision** to be made as to whether compliance with the standards of the monographs would be achieved if the official procedures were used. In the event of **doubt or dispute**, the analytical procedures of the Ph. Eur. are **alone authoritative**.



Pharmacopoeial procedure: roadmap

Basis for monograph elaboration

ICHQ2(R1) Validation of analytical procedures

Validated analytical procedure(s) (**approved specifications**)

Verification of analytical procedure(s)

Analytical procedure (re)validation (e.g., changes in operating conditions, adaptation to multi-source products)

Selection of suitable analytical procedure

Revision (new source on the market, analytical improvement)

Ph. Eur. general text *Comparability of alternative analytical procedures (5.27)*

Alternative analytical procedures

Pharmacopoeial procedure in monograph (description, operating conditions, parameters, reference standard(s), SST/performance controls)

General notices 1.1.2.5

Implementation (suitability under actual conditions of use: assessment, critical factors, verification experiments (APPCs*) / SST

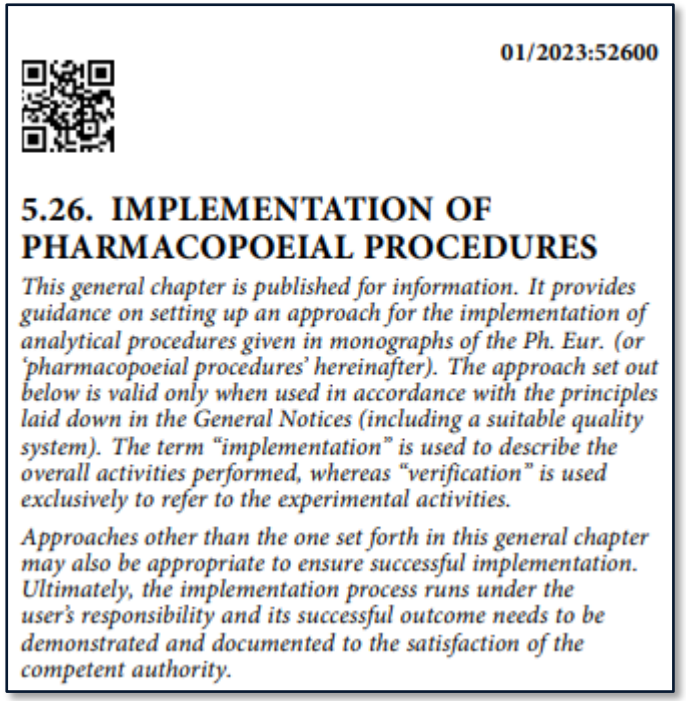
Ph. Eur. general text *Implementation of pharmacopoeial procedures (5.26)*

*APPC: analytical procedure performance characteristics (validation characteristics)

Routine use

Implementation of pharmacopoeial procedures (5.26)

- **General text published "for information"**
- **Guidance** on setting up an approach for implementation of analytical procedures given in Ph. Eur. monographs
- **Detailed information** on one of the key processes underpinning the correct usage of Ph. Eur. monographs
- Covers all types of analytical procedures given in Ph. Eur. monographs
- Approach described valid only when **used in accordance with** the principles laid down in the *General Notices* (including a suitable quality system)
- Other approaches may be appropriate.



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5.26. IMPLEMENTATION OF PHARMACOPOEIAL PROCEDURES

This general chapter is published for information. It provides guidance on setting up an approach for the implementation of analytical procedures given in monographs of the Ph. Eur. (or 'pharmacopoeial procedures' hereinafter). The approach set out below is valid only when used in accordance with the principles laid down in the General Notices (including a suitable quality system). The term "implementation" is used to describe the overall activities performed, whereas "verification" is used exclusively to refer to the experimental activities.

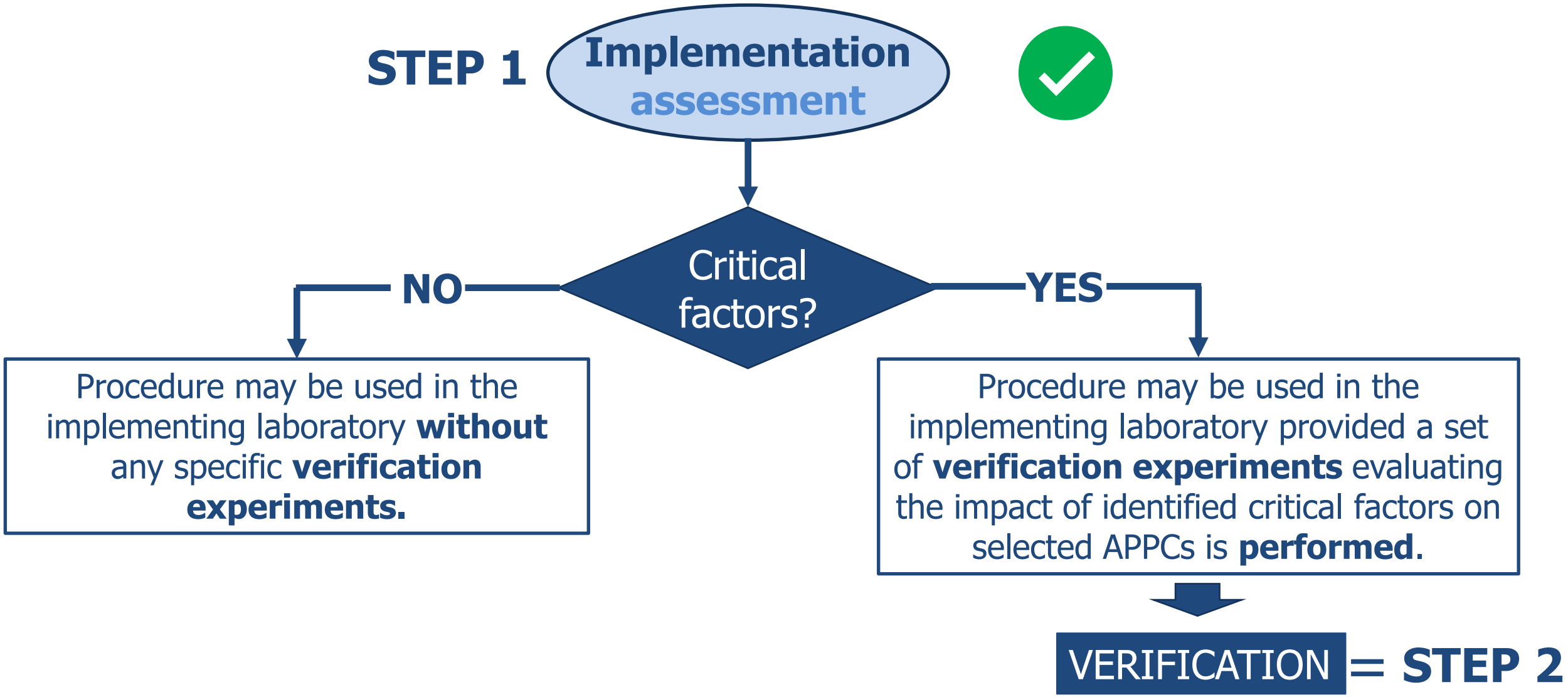
Approaches other than the one set forth in this general chapter may also be appropriate to ensure successful implementation. Ultimately, the implementation process runs under the user's responsibility and its successful outcome needs to be demonstrated and documented to the satisfaction of the competent authority.

General text 5.26: implementation process

STEP 1 - IMPLEMENTATION ASSESSMENT

- To identify any **critical factors** related to the actual conditions of use in the implementing laboratory that may affect the performance of the pharmacopoeial procedure:
  - composition of the article under test;
  - complexity of the sample preparation;
  - reagents required to run the procedure;
  - laboratory equipment required to run the procedure;
  - laboratory environment.
- Carried out in conjunction with provisions given in monographs and relevant general chapters, such as suitability requirements or any other performance tests described
- Two possible outcomes

General text 5.26: implementation process



General text 5.26: implementation process

STEP 2 - VERFICATION EXPERIMENTS

- To **demonstrate** that implementation is feasible
- **Relevant APPCs** are assessed and verified depending on the objective of the analytical procedure.

Verification plan

➢ experiments required to verify **critical APPCs** together with the corresponding acceptance criteria defined by the user

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5.26. IMPLEMENTATION OF PHARMACOPOEIAL PROCEDURES

Table 5.26.-1. – *Relevant APPCs to be recommended for verification based on the intended use of the procedure*

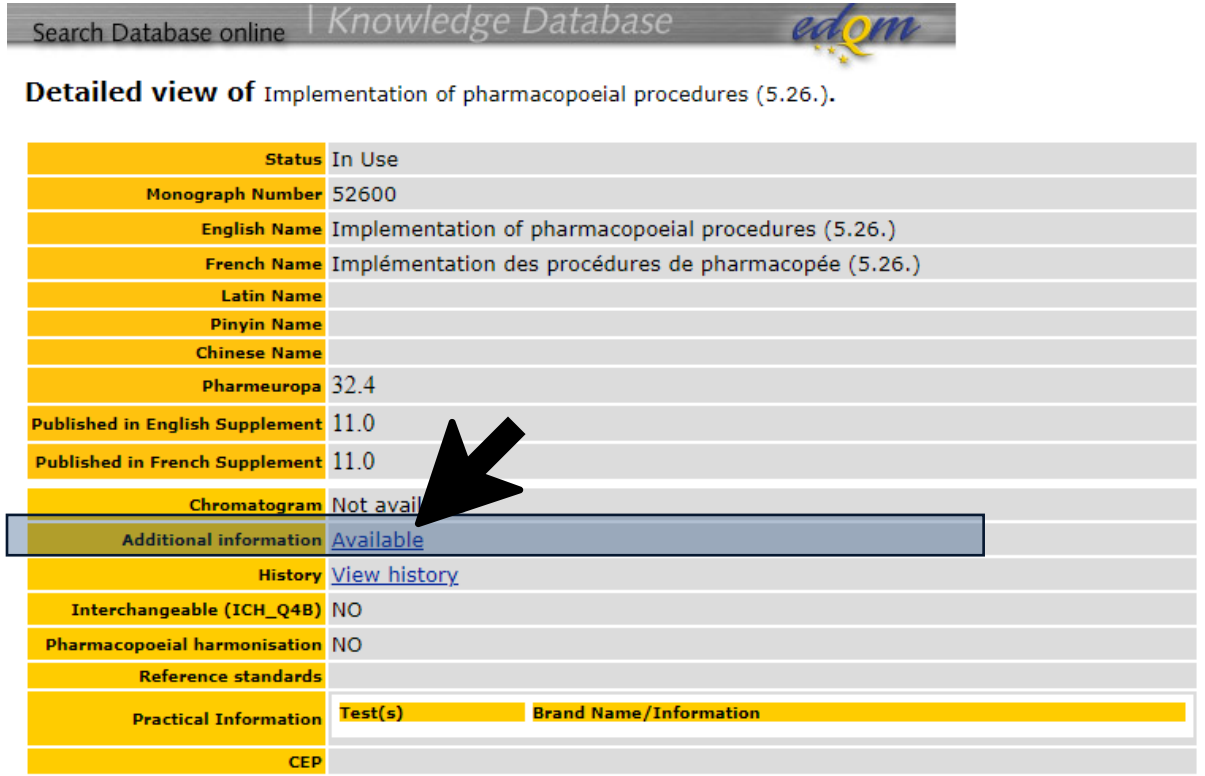
Intended use / APPCs	Identification	Testing for impurities		Assay - content/potency - dissolution (measurement only)	Other quantitative tests
		Limit test	Quantitative test		
Accuracy	○	○	○	◗	◗
Precision					
- Repeatability	○	○	●	●	●
- Intermediate precision	○	○	◗	◗	◗
Specificity/Selectivity	◗	●	●	●	◗
Sensitivity	○	●	●	○	◗
Linearity	○	○	○	◗	◗
Range	○	○	○	◗	◗
Robustness	○	○	◗	◗	◗

● *signifies that this characteristic should be experimentally verified.*
◗ *signifies that this characteristic should be experimentally verified, if impacted by critical factors from the actual conditions of use in the implementing laboratory.*
○ *signifies that this characteristic is typically not relevant for purposes of verification.*

Compliance with pre-defined acceptance criteria demonstrates that implementation of the pharmacopoeial procedure for a given article is feasible.

Implementation of pharmacopoeial procedures (5.26)

- **Examples of implementation** of pharmacopoeial procedures according to 5.26:
  - for **illustrative purposes** only
  - practical guidance on how to apply 5.26 concepts in concrete cases
  - "Ultimately, the **implementation process** runs under the **user's responsibility** and its successful outcome needs to be demonstrated and documented to the satisfaction of the competent authority."



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Detailed view of Implementation of pharmacopoeial procedures (5.26.).

Status	In Use
Monograph Number	52600
English Name	Implementation of pharmacopoeial procedures (5.26.)
French Name	Implémentation des procédures de pharmacopée (5.26.)
Latin Name	
Pinyin Name	
Chinese Name	
Pharmeuropa	32.4
Published in English Supplement	11.0
Published in French Supplement	11.0
Chromatogram	Not avail
Additional information	Available
History	View history
Interchangeable (ICH_Q4B)	NO
Pharmacopoeial harmonisation	NO
Reference standards	
Practical Information	Test(s) / Brand Name/Information
CEP	

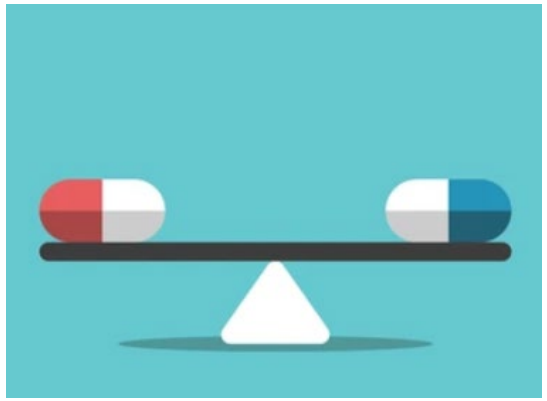
Implementation of pharmacopoeial procedures (5.26)

❑ Implementation examples

- **Identification by IR absorption spectrophotometry** (*0559, Mannitol (07/2019)*)
- **Related substances test by LC-UV** (*2986, Deferiprone tablets (01/2022)*)
- **Assay by LC-UV** (*0113, Benzylpenicillin potassium (07/2017;corrected 10.0*))
- **Potency by cell-based assay** (*2928, Infliximab concentrated solution (04/2023), Procedure B)*
- **Microbial enumeration** (*2987, Deferiprone oral solution (01/2021)*)
- **Sulfated ash** (*2236, Deferiprone (07/2018)*)

Elaboration of new general chapter 5.27: Rationale

- Need for guidance on how to demonstrate equivalency when the **official analytical procedure** (i.e., the pharmacopoeial procedure) is replaced by an alternative analytical procedure for control purposes.



Elaboration of a new general chapter on *Comparability of alternative analytical procedures* with the aim of providing practical guidance for alignment with the statement in *General Notices* **section 1.1.2.5.**

Online training

Webinar on new general chapter Comparability of alternative analytical procedures (5.27)

EUROPEAN PHARMACOPOEIA | 17/01/2024 | ON-DEMAND WEBINAR

SLIDO

- **I have another suitable (validated) analytical procedure that I consider superior to the pharmacopoeial procedure – can I replace the latter?**

✓No, I can solely use the pharmacopoeial procedure to analyse my product.

✓Yes, as I consider that my analytical procedure exceeds the required performance.

✓Yes, after demonstrating its comparability to the pharmacopoeial procedure.

Correct answer in green!

Key Aspects of general chapter 5.27

Framework

- Published for information
- Guidance on possible approaches
- No new requirements introduced
- 'Comparability' ≠ 'equality'

Scope

- Cases where a pharmacopoeial (official) analytical procedure, as referenced in an individual monograph, would be replaced by an alternative ("in-house") analytical procedure
- Applies to qualitative and quantitative analytical procedures

Not in scope

- Development of new analytical procedures
- Application of pharmacopoeial analytical procedures to articles not covered by Ph. Eur.

5.27. COMPARABILITY OF ALTERNATIVE ANALYTICAL PROCEDURES

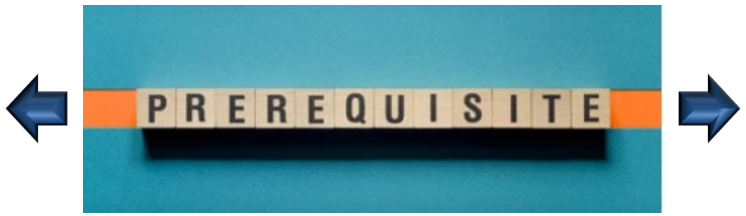
This general chapter is published for information. It
an alternative analytical procedure to a pharmacop
demonstrated. Other approaches to demonstrating c
The use of an alternative procedure is subject to autho
The final responsibility for the demonstration of compara
the successful outcome of the process needs to be demonstrated and documented to the satisfaction of the competent authority. Comparability must be maintained over the lifecycle of both the pharmacopoeial and alternative

General chapter 5.27: Preamble

This general chapter is published for information. It describes how the comparability of an alternative analytical procedure to a pharmacopoeial analytical procedure may be demonstrated. Other approaches to demonstrating comparability may also be appropriate. The use of an alternative procedure is subject to authorisation by the competent authority. The final responsibility for the demonstration of comparability lies with the user and the successful outcome of the process needs to be demonstrated and documented to the satisfaction of the competent authority. Comparability must be maintained over the lifecycle of both the pharmacopoeial and alternative analytical procedure.

Preliminary conditions

- **Alternative analytical procedure is validated** for its intended purpose in accordance with accepted scientific practice, current recommendations on analytical validation and guidelines that are relevant with regard to setting appropriate specification limits.



- **Pharmacopoeial procedure is implemented** as defined in general chapter *5.26. Implementation of pharmacopoeial procedures*, including verification experiments if appropriate.

Preliminary conditions: comparability assessment

Alternative analytical procedure (validated)

Pharmacopoeial procedure (implemented)

Comparison of analytical procedure performance

Demonstration that the alternative procedure meets its performance criteria during **validation** is not sufficient to imply comparability with pharmacopoeial procedure.

Comparability assessment of data generated during implementation of pharmacopoeial procedure and validation studies on alternative procedure:

- APPCs, such as **specificity/selectivity, sensitivity** (at the lower range limit), **linearity** and **range** should be assessed to ensure that the alternative procedure is at least as capable as the pharmacopoeial procedure
- Outcome of the comparability assessment may form the basis for the design of the *comparability study*

Comparability process

Step 1: Comparability assessment

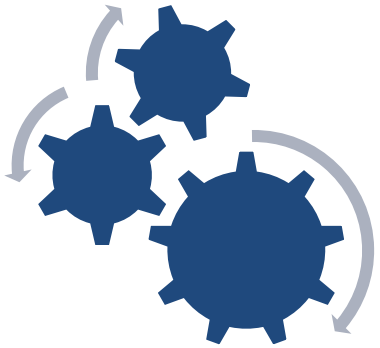
- Comparison of data obtained in the implementation of the pharmacopoeial procedure and validation data in terms of analytical procedure performance characteristics (APPCs)

Step 2: Comparability study

- **Head-to-head testing**, with the aim of reaching the same analytical decision
  → particularities: same experiments, same samples

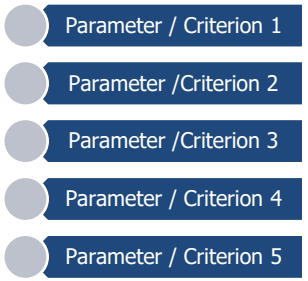
Study design

- Based on the outcome of the comparability assessment
- Considers special cases where testing in a head-to-head format is not feasible



Study protocol:

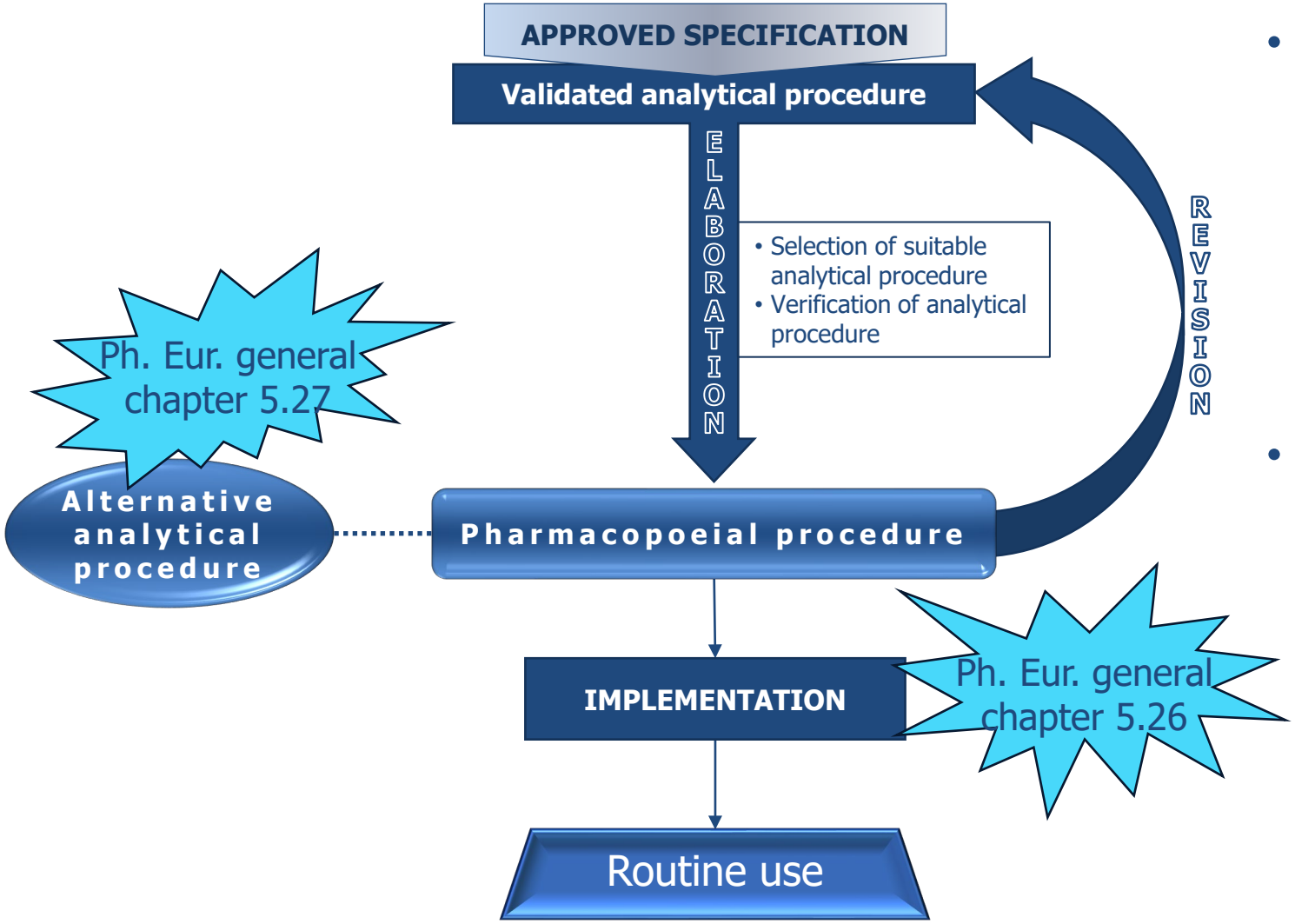
- established on the basis of the study design
- covers selection of samples and sample size, APPCs to be included and method for statistical evaluation of data
- includes definition of comparability through setting of **equivalence margin(s)** and **acceptance criteria** and their justification



Study report:

- summarises the results and conclusion of the comparability study, as well as other relevant information (e.g. deviations from study protocol, newly obtained information on the procedure(s) and or tested samples)

Lifecycle of the pharmacopoeial procedure



- If a user considers that the alternative analytical procedure brings a significant improvement for the quality of the article, they are encouraged to contact EDQM and/or submit a **request for revision** via their NPA
- In the event of an issue with a pharmacopoeial procedure (e.g. implementation difficulties), the EDQM should be contacted via the Helpdesk and if confirmed, this may result in a revision
  → **In itself not a case for an alternative procedure**

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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Module 1 Agenda

Ph. Eur. general concepts

Emmanuelle Charton, Mihaela Buda, EDQM, Council of Europe

Biotherapeutics

Mihaela Buda, EDQM, Council of Europe

Advanced therapy medicinal products (ATMPs)

Olga Kolaj-Robin, EDQM, Council of Europe

Ph. Eur. Texts on Biotherapeutics

Module 1: General concepts. Biotherapeutics and ATMPs

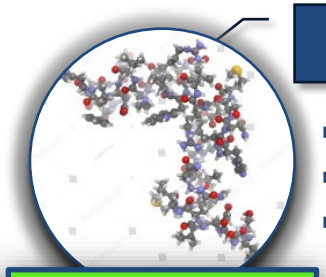
30 January 2024

Mihaela Buda, PhD
European Pharmacopoeia Department
EDQM, Council of Europe

Presentation Outline

- **Biotherapeutics – Ph. Eur. monograph portfolio**
  - General aspects
- **General monographs**
  - Monoclonal antibodies for human use – visible particles
- **Individual monographs**
  - A guide through monographs on rDNA proteins
  - Flexibility to address complexity
  - Case studies
- **General chapters** supporting individual monographs on biotherapeutics (examples)
- Strategy for Ph. Eur. quality standards for multi-source monoclonal antibodies: monographs and horizontal standards

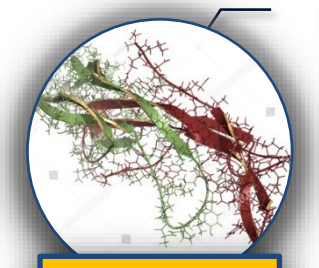
Biotherapeutics – Ph. Eur. Monograph Portfolio



3000-4000 Da

Peptides

- Calcitonin salmon (0471)
- Glucagon, human (1635)
- Teriparatide (2829)



20-40 KDa

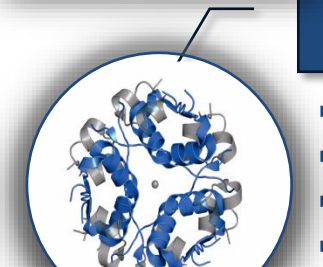
Hormones, growth factors

- Somatropin concentrated solution (0950)
- Somatropin (0951)
- Somatropin for injection (0952)*
- Somatropin solution for injection (2370)*
- Erythropoietin conc. solution (1316)
- Follitropin (2285)
- Follitropin concentrated solution (2286)

~150 KDa

Fusion proteins

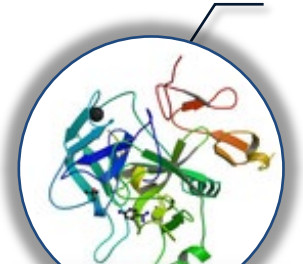
- Etanercept (2895)



5000-6000 Da

Insulins and analogues

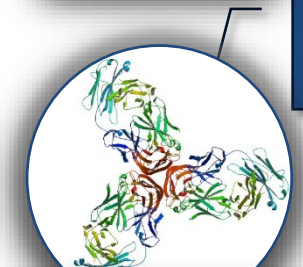
- Insulin aspart (2084)
- Insulin glargine (2571)
- Insulin lispro (2085)
- Insulin preparations injectable (0854)*
- Insulin, human (0838)



50-65 kDa

Clotting factors, TPA

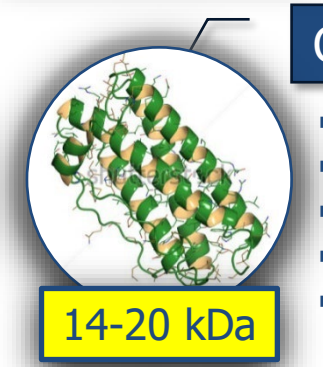
- Alteplase for injection (1170)
- Human coagulation factor IX (rDNA) powder for solution for injection (2994)*
- Human coagulation factor IX rDNA concentrated solution (2522)
- Human coagulation factor VIIa rDNA concentrated solution (2534)



~150 KDa

Monoclonal antibodies

- Infliximab concentrated solution (2928)
- *Golimumab concentrated solution (3103)*



14-20 kDa

Cytokines, interferons

- Filgrastim concentrated solution (2206)
- Filgrastim injection (2848)
- Interferon alfa-2 concentrated solution (1110)
- Interferon gamma-1b concentrated solution (1440)
- Molgramostim concentrated solution (1641)

>200 KDa

Clotting factors

- Human coagulation factor VIII rDNA (1643)*

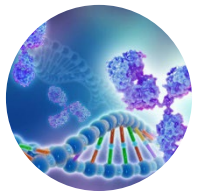
* medicinal product monographs

New monographs in preparation – see here

Biotherapeutics – Ph. Eur. monograph portfolio

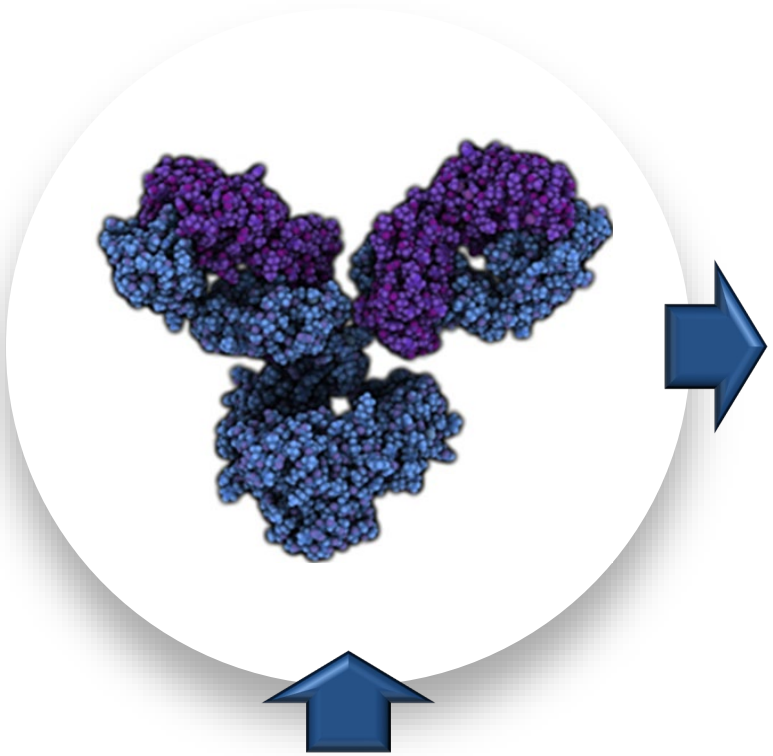
General monographs

- Dosage form monographs
- Pharmaceutical preparations (2619)
- Substances for pharmaceutical use (2034)
- Products with risk of transmitting agents of animal spongiform encephalopathies (1483)



- Recombinant DNA technology products of (0784)
- **Monoclonal antibodies for human use (2031)**

Monoclonal Antibodies for Human Use (2031)



General requirements for:

- Active substance, final bulk, final lot
- **Medicinal product**:
  - **Visible particles**
  - Molecular identity and structural integrity
  - Molecular-size distribution
  - Purity .....

- **Parenteral preparations (0520)**
  - Eye preparations (1163)

Dosage form monographs

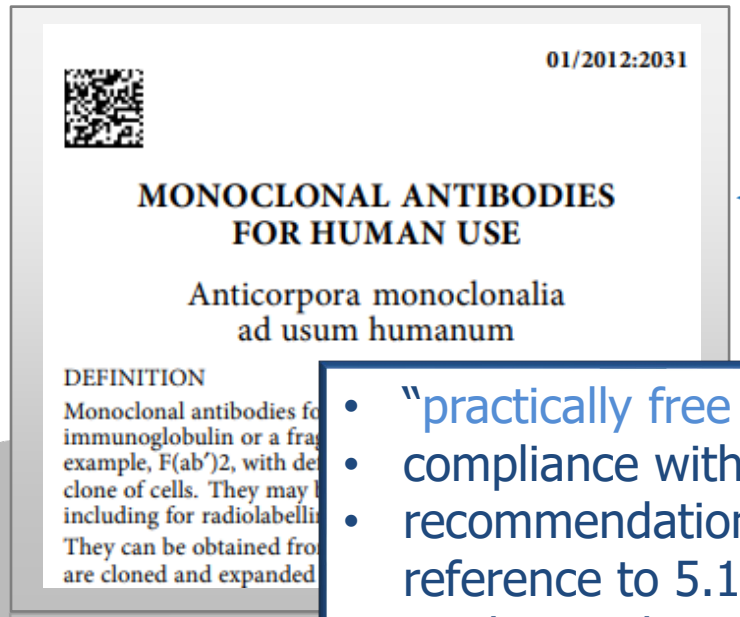
Online training

Webinar: Data Particulate contamination in parenteral preparations: what's new in the Ph. Eur.? Are monoclonal antibodies a special case?

EUROPEAN PHARMACOPOEIA | 14/12/2021 | ON-DEMAND WEBINAR

Particulate Contamination: Monograph Alignment

2031 **Monoclonal antibodies for human use**



01/2012:2031

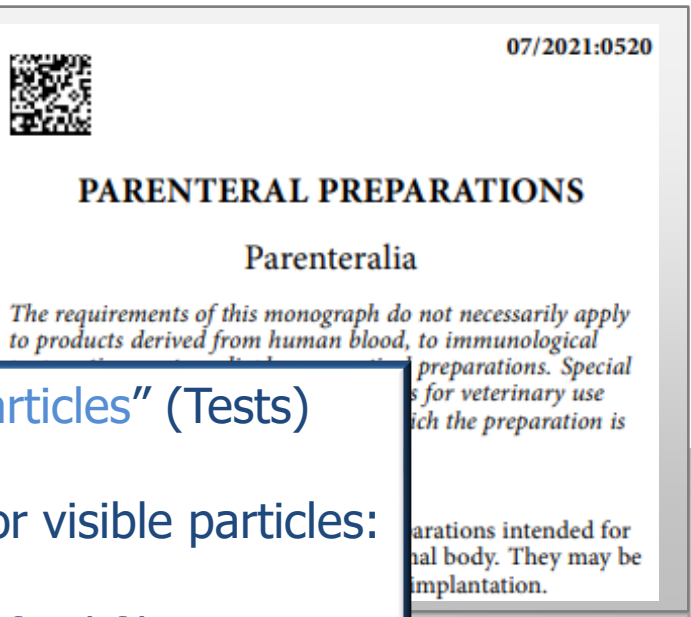
MONOCLONAL ANTIBODIES FOR HUMAN USE

Anticorpora monoclonalia ad usum humanum

DEFINITION

Monoclonal antibodies fo
immunoglobulin or a frag
example, F(ab')2, with de
clone of cells. They may
including for radiolabelli
They can be obtained fro
are cloned and expanded

0520 **Parenteral preparations**



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PARENTERAL PREPARATIONS

Parenteralia

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations. Special for veterinary use ich the preparation is

arations intended for
al body. They may be
implantation.

- "practically free from visible particles" (Tests)
- compliance with 2.9.20
- recommendations on testing for visible particles: reference to 5.17.2
- products administered using a final filter

✓ **Revised monograph published in Ph. Eur. 11.2 (07/2023)**

✓ **Revised monograph published in Ph. Eur. 10.5 (07/2021)**

Monoclonal Antibodies for Human Use (2031) (1/2)

Revised monograph - Particles

- **PRODUCTION section** [*FINAL LOT*]
  - Liquid preparations for injection or infusion, examined under suitable conditions of visibility, are **practically free from particles**
  - Recommendations on testing for visible particles and **reference to new general chapter 5.17.2**

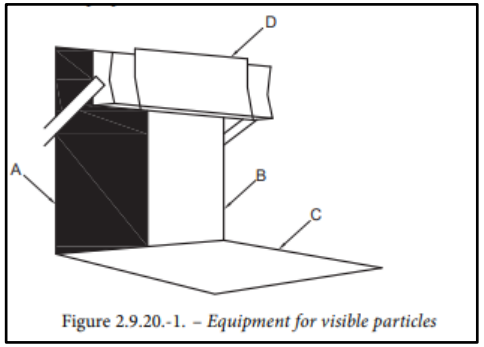


Figure 2.9.20.-1. – *Equipment for visible particles*

Parenteral preparations (0520)

Monoclonal Antibodies for Human Use (2031) (2/2)

Revised monograph - particles

- **TESTS. Appearance**
  - **Compliance with general chapter 2.9.20**
  - "Without visible particles" replaced with "**practically free from visible particles**":
    - "**unless otherwise justified and authorised**" intentionally kept for cases in which manufacturers can demonstrate that it is not possible to remove all visible particles, due to the inherent nature of monoclonal antibodies;
  - Recommendations added on testing for visible particles and **reference to new general chapter 5.17.2**
  - Specific provisions added for **products administered using a final filter**, as stated on the label.

'**Practically free from visible particles**' reflects the capability of the manufacturing and testing process. The term is applicable at the batch level of a medicinal product, not for single units examined individually. However unrealistic, a 'zero particles' product is nonetheless a worthy goal. " **(5.17.2)**

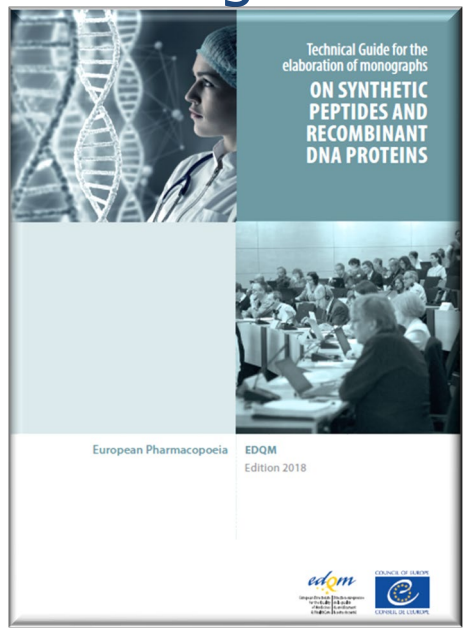
'**Unless otherwise justified and authorised**'. This expression means that the requirements must be met, unless the competent authority authorises a modification (e.g. of an analytical procedure or limit) or an exemption, if justified by the manufacturer in a particular case.
(Ph. Eur. General Notices)

Products administered using a filter
With some parenteral products, for example products for which there is insufficient product knowledge, filters may be used to reduce the risks related to particles that may form during **storage**. However, the use of such filters does not constitute acceptance of particles after manufacture or allow particulate contamination per se. If justified and authorised, products administered using a filter can be exempt from the 'practically free from particles' requirement, providing it has been demonstrated that the filter delivers a filtrate that complies. **(5.17.2)**

A Guide Through Individual Monographs on rDNA Proteins

Ph. Eur. Monograph Elaboration: General Principles

- **Monograph specifications** are based on those of medicinal products currently approved by member states unless otherwise agreed by the EPC (e.g. in the case of unlicensed medicinal products)
- Approved specification(s) are the main basis for monograph elaboration, backed up by batch data
- **Analytical procedures** included in monographs are validated according to current guidelines
- All individual monographs are verified experimentally
- Draft monographs are **reviewed by stakeholders/users** including regulatory authorities, at Pharmeuropa stage
- Policy for monograph development is given in **technical guides** (available on the EDQM website)



Individual Monographs for Biotherapeutics

Public standard

One part of a total control strategy designed to ensure product quality and consistency

A set of relevant quality attributes, in order to confirm the quality of the active substance in question

It is not its aim to go beyond to characterisation that includes determination of physicochemical and immunochemical properties, biological activity, purity and impurities, an extensive program conducted by a combination of orthogonal methodologies

Active Substance Monographs for rDNA Proteins

MONOGRAPH SECTION

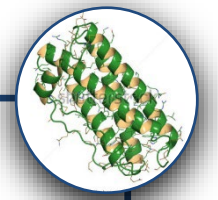
Version date

Title
INN

Formula
molecular and graphic

Relative mass
glycosylated protein except for complex glycoproteins

CAS number



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corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

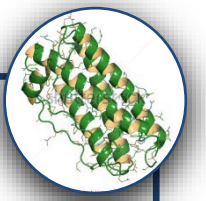
Filgrastimi solutio concentrata

MTPLGPASSL PQSFLLKCLE QVRKIQGDGA ALQEKLCATY
KLCHPEELVL LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH
SGLFLYQGLL QALEGISPEL GPTLDTLQLD VADFATTIWQ
QMEELGMAPA LQPTQGAMPA FASAFQRRAG GVLVASHLQS
FLEVSYRVLR HLAQP

$C_{845}H_{1339}N_{223}O_{243}S_9$ M_r 18 799
[121181-53-1]

Active Substance Monographs for rDNA Proteins

MONOGRAPH SECTION



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FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

➢ Definition

- chemical nomenclature
- identity and biological activity
- physical form
- assay limits:
  - protein content (mass/volume or mass/mass)
  - specific activity (IU/mg); by convention in some cases (e.g. somatropin, insulin)

➡

DEFINITION

Solution of a protein having the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (huG-CSF) plus 1 additional amino acid, an *N*-terminal methionine. In contrast to its natural counterpart, the protein is not glycosylated. huG-CSF is produced and secreted by endothelial cells, monocytes and other immune cells. The protein stimulates the differentiation and proliferation of leucocyte stem cells into mature granulocytes.

Content: minimum 0.9 mg of protein per millilitre.

Potency: minimum 0.9×10^8 IU per milligram of protein.

Active Substance Monographs for rDNA Proteins

MONOGRAPH SECTION

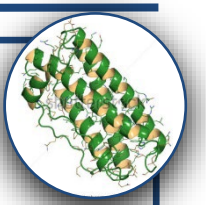
➢ Production

- Always present for biologicals
- extensive for vaccines
- may contain specific tests for rDNA products
- source materials, manufacturing process, validation, control, in-process testing
- mandatory requirements for manufacturers
- independent verification difficult
- compliance: competent authorities

➢ Characters*

- appearance, hygroscopicity, crystallinity, solubility
- useful info for analyst
- not analytical requirement

* See also 5.11 Characters section in monographs



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FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(…)

PRODUCTION

Filgrastim concentrated solution is produced by a method based on recombinant DNA (rDNA) technology, using bacteria as host cells.

Prior to release, the following tests are carried out on each batch of filgrastim concentrated solution, unless exemption has been granted by the competent authority.

Host-cell-derived proteins. The limit is approved by the competent authority.

Host-cell- or vector-derived DNA. The limit is approved by the competent authority.

CHARACTERS

Appearance: clear, colourless or slightly yellowish liquid.

Active Substance Monographs for rDNA Proteins

MONOGRAPH SECTION

➢ Identification

- substance-specific, based on unique aspects of substance's molecular structure and/or other specific properties
- often cross-references to *Tests* and *Assay*
- confirmation of molecule's:
  - size
  - sequence
  - isoelectric profile
  - chromatographic properties
  - correct functional configuration
  - specific to product (*e.g.* glycan analysis)

(...)

IDENTIFICATION

A. It shows the expected biological activity (see Assay).

B. Examine the electropherograms obtained in the test for impurities with charges differing from that of filgrastim. *Results*: the principal band in the electropherogram obtained with the test solution is similar in position to the principal band in the electropherogram obtained with reference solution (a).

C. Examine the chromatograms obtained in the test for impurities with molecular masses higher than that of filgrastim.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

D. Examine the electropherograms obtained under both reducing and non-reducing conditions in the test for impurities with molecular masses differing from that of filgrastim.

Results: the principal band in the electropherogram obtained with test solution (a) is similar in position to the principal band in the electropherogram obtained with reference solution (b).

E. Examine the chromatograms obtained in the test for related proteins.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and shape to the principal peak in the chromatogram obtained with the reference solution.

F. Peptide mapping (*2.2.55*).

Active Substance Monographs for rDNA Proteins

MONOGRAPH SECTION

➢ Tests

- **Purity/impurity assessment**
- Developed on basis of protein size, charge and hydrophobicity
- **Specific procedures** for detection and quantification of specific impurities if necessary
- **Limits based on specifications** and batch data for approved products
- Bacterial endotoxins – covered by 2034; may not be repeated)
- Residual solvents – covered by 2034
- Inorganic impurities e.g. sulphated ash

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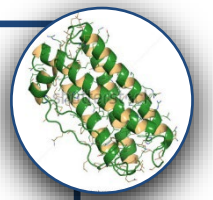

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

(...)

Impurities with molecular masses differing from that of filgrastim. Polyacrylamide gel electrophoresis (*2.2.31*) under both reducing and non-reducing conditions.

(...)

Impurities with charges differing from that of filgrastim. Isoelectric focusing (*2.2.54*).

(...)

Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure.

(...)

Bacterial endotoxins (*2.6.14*): less than 2 IU in the volume that contains 1.0 mg of protein.

Active Substance Monographs for rDNA Proteins

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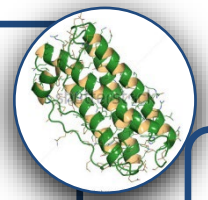


FILGRASTIM CONCENTRATED SOLUTION

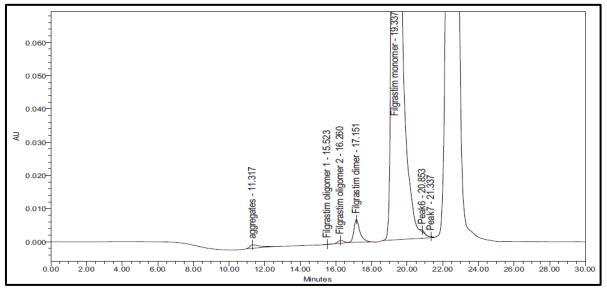
Filgrastimi solutio concentrata

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

System suitability: resolution solution:
- *retention time*: filgrastim monomer = 17 min to 20 min;
- *resolution*: minimum 3 between the peaks due to the filgrastim dimer and the filgrastim monomer.

Calculate the percentage content of the dimer, oligomers and aggregates.

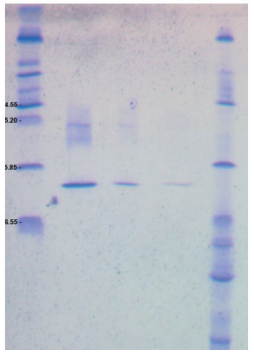
Limits:
- *impurities with molecular masses higher than that of filgrastim, other than the dimer*: maximum 0.5 per cent;
- *total of impurities with molecular masses higher than that of filgrastim*: maximum 2 per cent.



Impurities with charges differing from that of filgrastim. Isoelectric focusing (*2.2.54*).

System suitability:
- in the electropherogram obtained with reference solution (c), the relevant isoelectric point markers are distributed along the entire length of the gel;
- in the electropherogram obtained with reference solution (a), the pI of the principal band is 5.7 to 6.3.

Limit:
- *any impurity*: no band is more intense than the principal band in the electropherogram obtained with reference solution (b) (10 per cent).

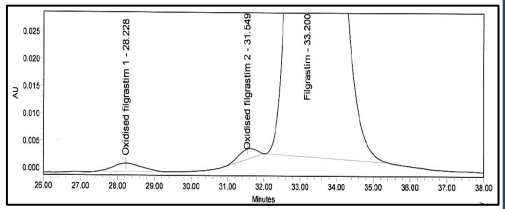


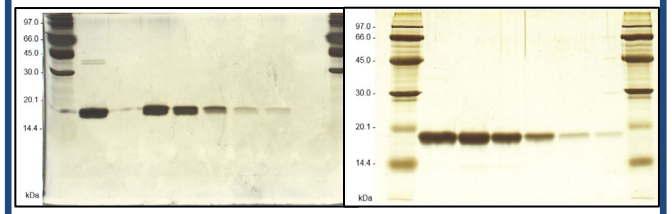
Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure.

System suitability: reference solution (b):
- *symmetry factor*: maximum 1.8 for the peak due to filgrastim;
- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to oxidised filgrastim (form 2) and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to filgrastim.

System suitability: reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to filgrastim and reduced filgrastim;
- *symmetry factor*: maximum 1.8 for the peak due to filgrastim.

Limits:
- *any impurity*: for each impurity, maximum 1.0 per cent;
- *total*: maximum 2.0 per cent.





Impurities with molecular masses differing from that of filgrastim. Polyacrylamide gel electrophoresis (*2.2.31*) under both reducing and non-reducing conditions.

System suitability:
- reference solution (a): the validation criteria are met;
- a band is seen in the electropherogram obtained with test solution (e);
- a gradation of intensity of staining is seen in the electropherograms obtained with test solutions (a) to (e).

Limit: test solution (a):
- *impurities with molecular masses lower or higher than that of filgrastim*: no band is more intense than the principal band in the electropherogram obtained with test solution (d) (2.0 per cent).

Active Substance Monographs for rDNA Proteins

MONOGRAPH SECTION

- **Assay**
  - protein content (comparative LC, UV spectroscopy)
  - bioassay with ref. to WHO IS or Ph. Eur. standard calibrated in IU
  - exceptionally: *in vivo* tests; physicochemical tests only; example procedures

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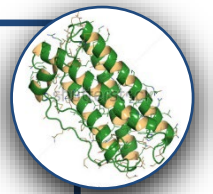
corrected 11.0



FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

ASSAY

Protein. Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection: test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency. The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the International Standard of filgrastim or with a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

(...)

Calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel line assay (*5.3*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

SLIDO

- **My filgrastim concentrated solution preparation fulfils the requirements for protein content and estimated potency described in Assay. Can potency be considered addressed?**

  - ✓ Yes
  - ✓ No
  - ✓ I don’t know

Correct answer in green!

Active Substance Monographs for rDNA Proteins

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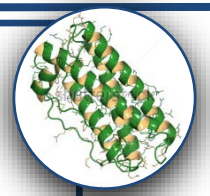
corrected 11.0



FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(…)

ASSAY

Protein. Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection: test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency. The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the International Standard of filgrastim or with a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

(…)

Calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel line assay (*5.3*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

DEFINITION

(…)

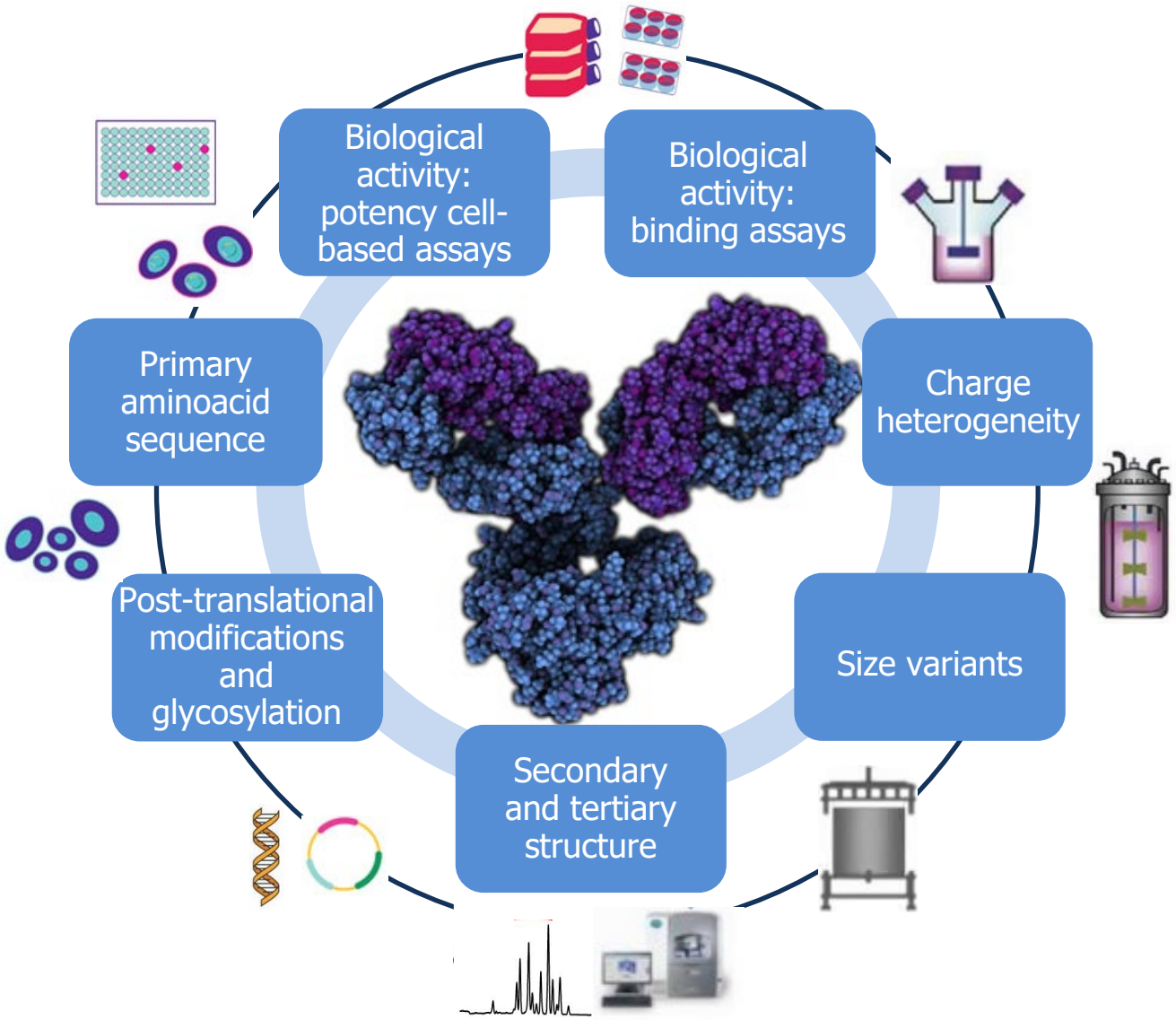
Content: minimum 0.9 mg of protein per millilitre.

Potency: minimum 0.9×10^8 IU per milligram of protein.

specific activity

Lot	Stated potency	Estimated potency	Protein content	Potency (specific activity)
1	1.0 x 10^8 IU/ml	0.8 x 10^8 IU/ml	1.0 mg/mL	0.8 x 10^8 IU/mg ✗
2	1.0 x 10^8 IU/ml	1.25 x 10^8 IU/ml	1.25 mg/mL	1.0 x 10^8 IU/mg ✓
3	1.0 x 10^8 IU/ml	1.0 x 10^8 IU/ml	1.25 mg/mL	0.8 x 10^8 IU/mg ✗

Complexity of Biotherapeutics



Challenge for setting monograph specifications

Ph. Eur. Monographs for Biotherapeutics

Monograph specifications

- Flexibility of expectations, so that they apply to a large variety of products:
  - **Ph. Eur. General Notices** (alternative procedures; waiving of tests; enhanced approaches)
  - **"Additional" flexibility**
- Prescriptive requirements so that the respective test procedures can be applied successfully in a control laboratory/allow independent testing:
  - detailed analytical procedures; SST criteria; Ph. Eur. standards
  - acceptance criteria for quality attributes

MONOGRAPH FLEXIBILITY

Additional Flexibility

Production section (Ph. Eur. General Notices)

- Requirements related to process-dependent heterogeneity **set in a flexible way** (*e.g.* glycan profile, charged variants)

Test procedures

- Generic methods of analysis (*e.g.* developed according to general chapters) – **suitable** procedures
- Specific analytical procedures – '**example**' procedure

Acceptance criteria for quality attributes

- Numeric limits/ ranges (specific activity; primary structure; related proteins; HMW species)
- 'As authorised by the competent authority' (process-dependent quality attributes)

Reference preparations

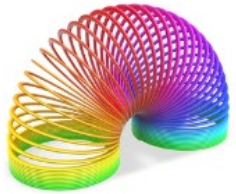
- Ph. Eur. reference standards for SST
- *In-house reference preparation* – for comparative purpose (*e.g.* matching LC profiles)

Monograph flexibility

Individual monographs can address complexity of biotherapeutics

Ph. Eur. Monographs for Biotherapeutics: Flexibility (2)

Test procedures: "Suitable" / "Example"



SUITABLE PROCEDURE

- **general indications** on the test procedure (main steps to be carried out, type of method, readout, cells, reagents…)
- the term "suitable" is a **conventional term:** '*In certain monographs […], the terms 'suitable' and 'appropriate' are used to describe a reagent, micro-organism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.*'

EXAMPLE PROCEDURE

- **specific instructions**, quantities, concentrations, compositions of reagents/buffers, chromatographic conditions etc. together with **system suitability criteria**

⇨ "*The following procedure is given as an example.*"

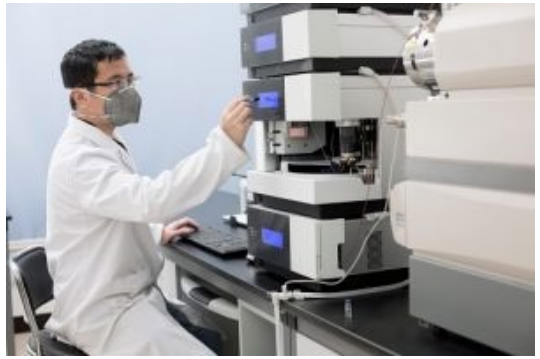
Terminology defined in Ph. Eur. General Notices

Flexibility: Example procedure

✓ The "example" analytical procedure has been validated for the intended purpose

A

Implementation*
& use the example procedure



OR

B

Replacement by another suitable **validated** procedure

No need to demonstrate equivalence to the procedure in the monograph

subject to approval by the competent authority.

* As defined in Ph. Eur. General Notices and further explained in general chapter 5.26

Case Study 1: Erythropoietin Monograph – Production

Production section

General requirements for **consistency of production**

Erythropoietin is produced in rodent cells *in vitro* by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process consistently produces a product with the expected glycosylation pattern using suitably qualified assay(s).

Requirements for **process-related impurities** derived from the upstream process

Host-cell-derived proteins. The limit is approved by the competent authority.

Host-cell and vector-derived DNA. The limit is approved by the competent authority.

Specific requirements related to **process-dependent heterogeneity**

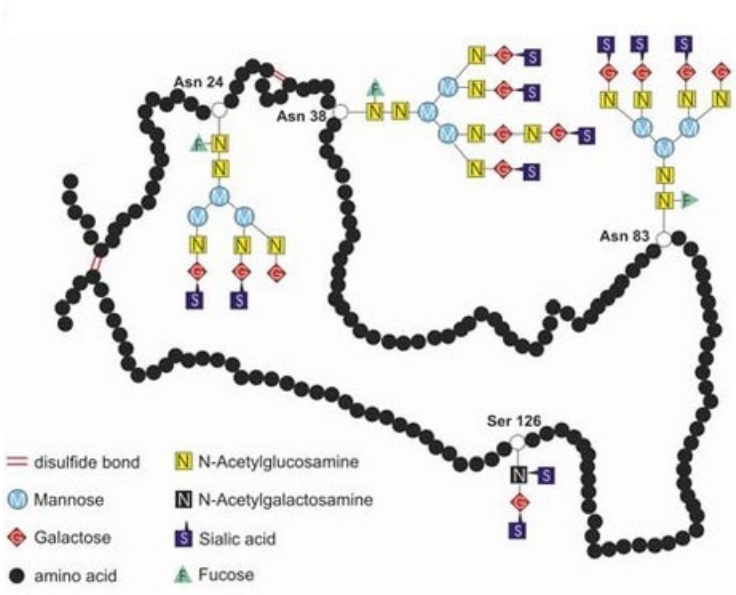
Generic method

***N*-Glycan analysis.** Use a suitable method developed according to general chapter *2.2.59. Glycan analysis of glycoproteins*, section 2-3:

- release the glycans using one of the agents described in Table *2.2.59.*-1, for example peptide *N*-glycosidase F (PNGase F);
- label the released glycans with one of the fluorescent labelling agents described in Table *2.2.59.*-2, for example 2-aminobenzamide;
- analyse the labelled glycans by liquid chromatography (*2.2.29*) using fluorescence detection.

Specific procedure as **example**

The following procedure is given as an example.



3 *N*-glycosylation sites (Asn-24, Asn-38, Asn83)
1 *O*-glycosylation site (Ser-126)

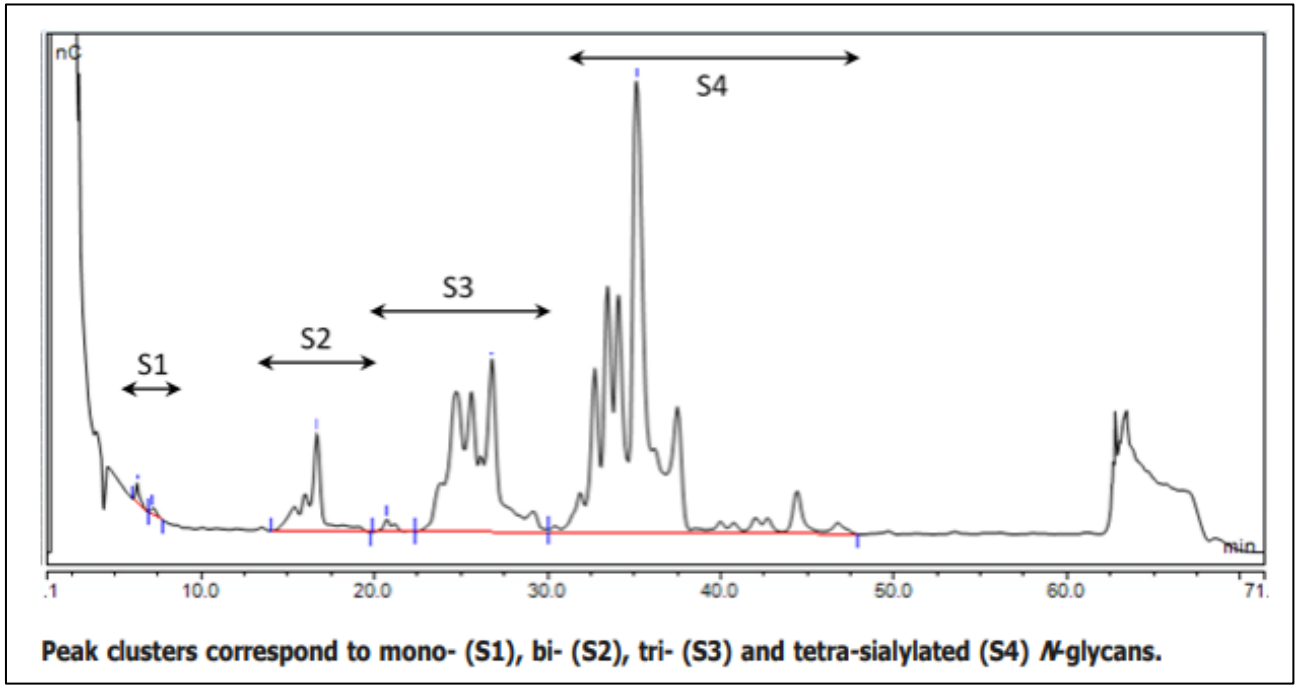
M_r approx. 30 600 Da

Case Study 1: Erythropoietin Monograph – Production

N-glycan analysis: specific procedure as example

- Detailed **description**:
  - **sample preparation**
  - PNGase **digestion**
  - **LC analysis** (electrochemical detection): HPAEC-PAD chromatographic conditions, mobile phase, gradient, separation conditions; SST

- *Identification of peaks*: use the chromatogram supplied with *erythropoietin for physicochemical tests CRS* to identify the 4 peak clusters corresponding to mono- (S1), bi- (S2), tri- (S3) and tetra-sialylated (S4) N-glycans.



Peak clusters correspond to mono- (S1), bi- (S2), tri- (S3) and tetra-sialylated (S4) *N*-glycans.

Case Study 1: Erythropoietin Monograph

N-glycan analysis: reference preparations

Reference solution (a): Erythropoietin for physicochemical tests CRS

System suitability:

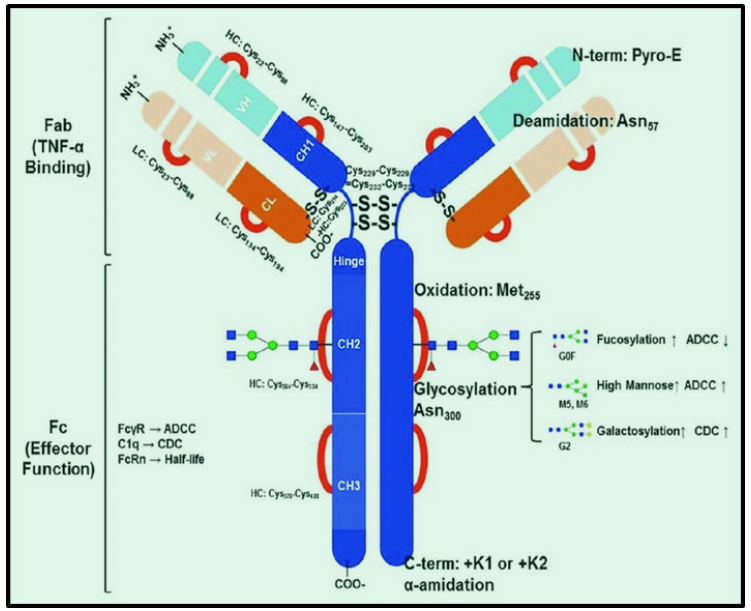
- the chromatogram obtained with reference solution (a) is qualitatively similar to the chromatogram supplied with *erythropoietin for physicochemical tests CRS*.

Reference solution (b): a suitable etanercept in-house reference preparation [...]

Results:

- the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with reference solution (b);
- the retention times of the peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with reference solution (b);
- no additional peaks are observed in the chromatogram obtained with the test solution in comparison with the chromatogram obtained with reference solution (b).

Case Study 2: Infliximab Monograph – Acceptance Criteria



$C_{6462}H_{9960}N_{1728}O_{2036}S_{44}$
M_r approx. 145 kDa (without glycosylation)

Quality attribute	Monograph specifications	
	Test procedure	Acceptance criteria
Protein content	see Assay (protein)	✓
Potency (specific activity)	see Assay (protein and potency)	×
Host-cell-derived proteins	Ph. Eur. 0784; 2.6.34	✓
Host-cell- and vector-derived DNA	Ph. Eur. 0784; *2.6.35*	✓
Residual protein A	Ph. Eur. 2.7.1	✓
Glycan analysis	Ph. Eur. 2.2.59; Example procedure	✓
Charged variants (acidic and basic variants)	**A. IEF** (Ph. Eur. 2.2.54); Example procedure *Alternative method: capillary IEF*	✓
	B. CEX-HPLC	✓
Peptide mapping (primary structure)	Trypsin digestion	×
pH	Ph. Eur. 2.2.3	✓
Related proteins (fragmentation)	CE-SDS reducing and non-reducing	×
HMW and LMW species	SEC	×
Protein	UV determination	-
Potency (Fab-related) biological activity	TNF-α neutralisation Example procedure; suitable procedures	- ×

SLIDO

- **Flexibility in a Ph. Eur. monograph for a complex biotherapeutic is**:

  - ✓ Allowed by the Ph. Eur. General Notices.
  - ✓ Built into its Production section.
  - ✓ Provided by analytical procedures given as examples.
  - ✓ Provided by reference to limits approved by the competent authority.
  - ✓ Built into its Identification and Tests section.
  - ✓ Provided by the use of in-house reference preparations in all test procedures included in the monograph.

Correct answers in green!

General Chapters (Methods of Analysis) Supporting Individual Monographs on Biotherapeutics

Overview of Applicable General Chapters

Mass spectrometry (2.2.43)

Statistical analysis of results of biological assays and tests (5.3)

Capillary electrophoresis (2.2.47)

Absorption spectrophotometry, UV-Vis (2.2.25)

Electrophoresis (2.2.31)

Peptide mapping (2.2.55)

Total protein (2.5.33)

Immunochemical methods (2.7.1)

Isoelectric focusing (2.2.54)

Size-exclusion chromatography (2.2.30)

Liquid chromatography (2.2.29)

Chromatographic separation techniques (2.2.46)

Amino acid analysis (2.2.56)

Glycan analysis of glycoproteins (2.2.59)

NEW

Quantification and characterisation of residual host-cell DNA (2.6.35)

Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)

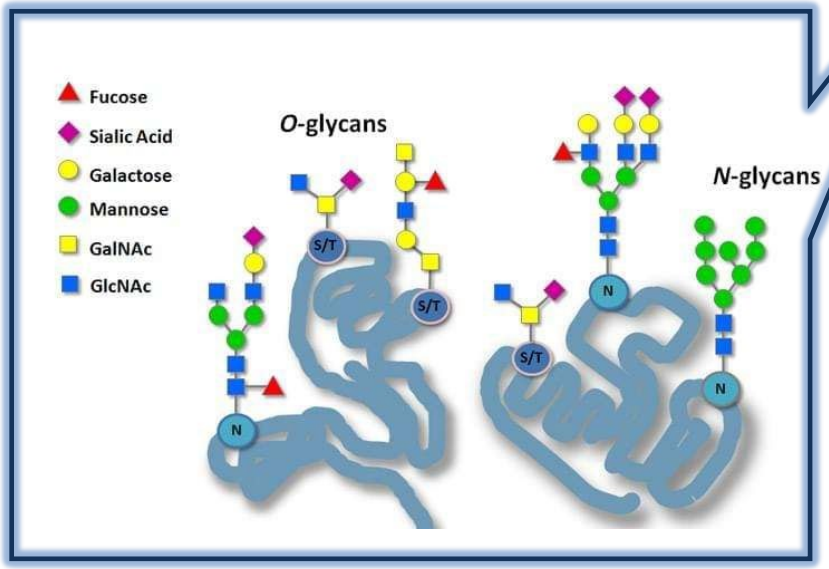
Host-cell protein assays (2.6.34)

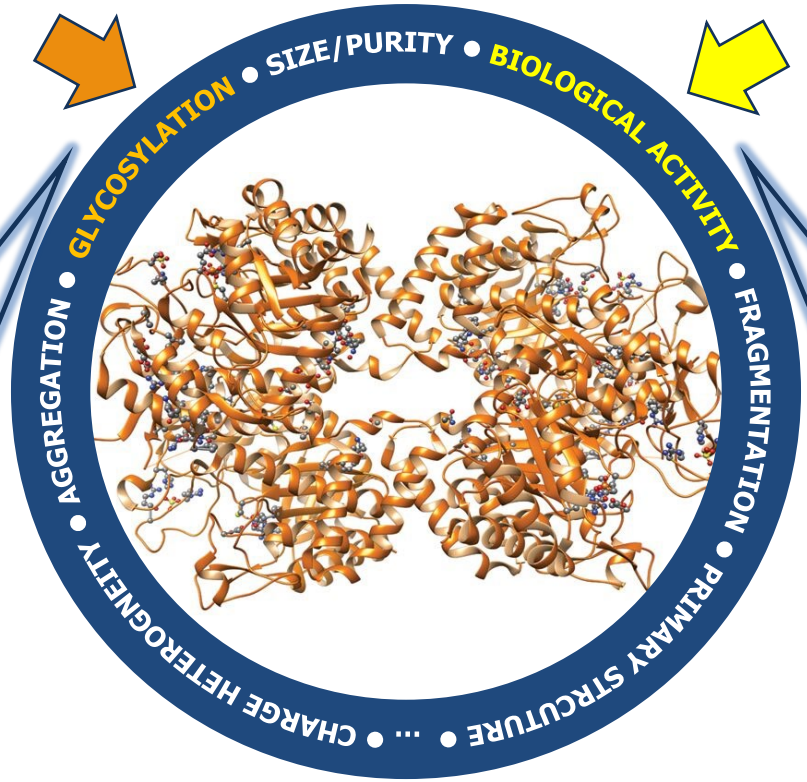
Non exhaustive list (...)

Tools for Analytical Characterisation/Quality Control

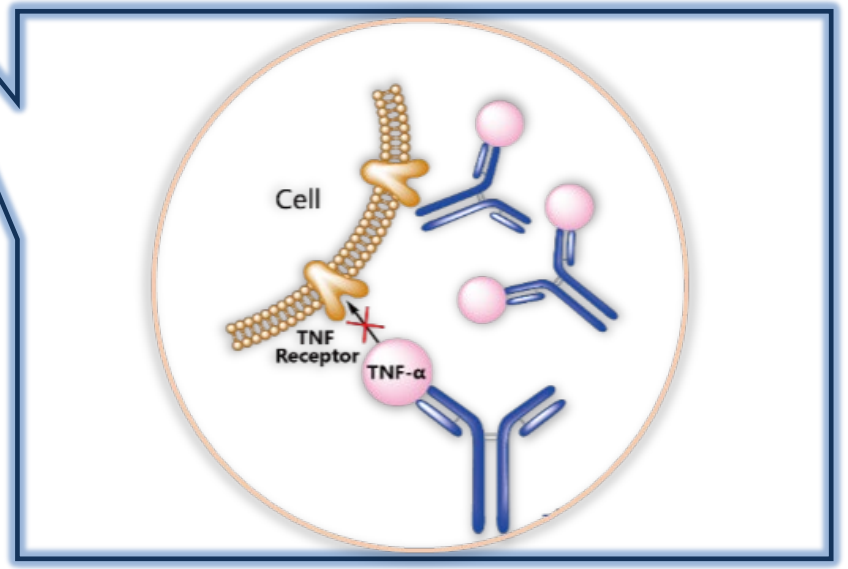
Examples

Glycan analysis of glycoproteins (2.2.59)





Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)

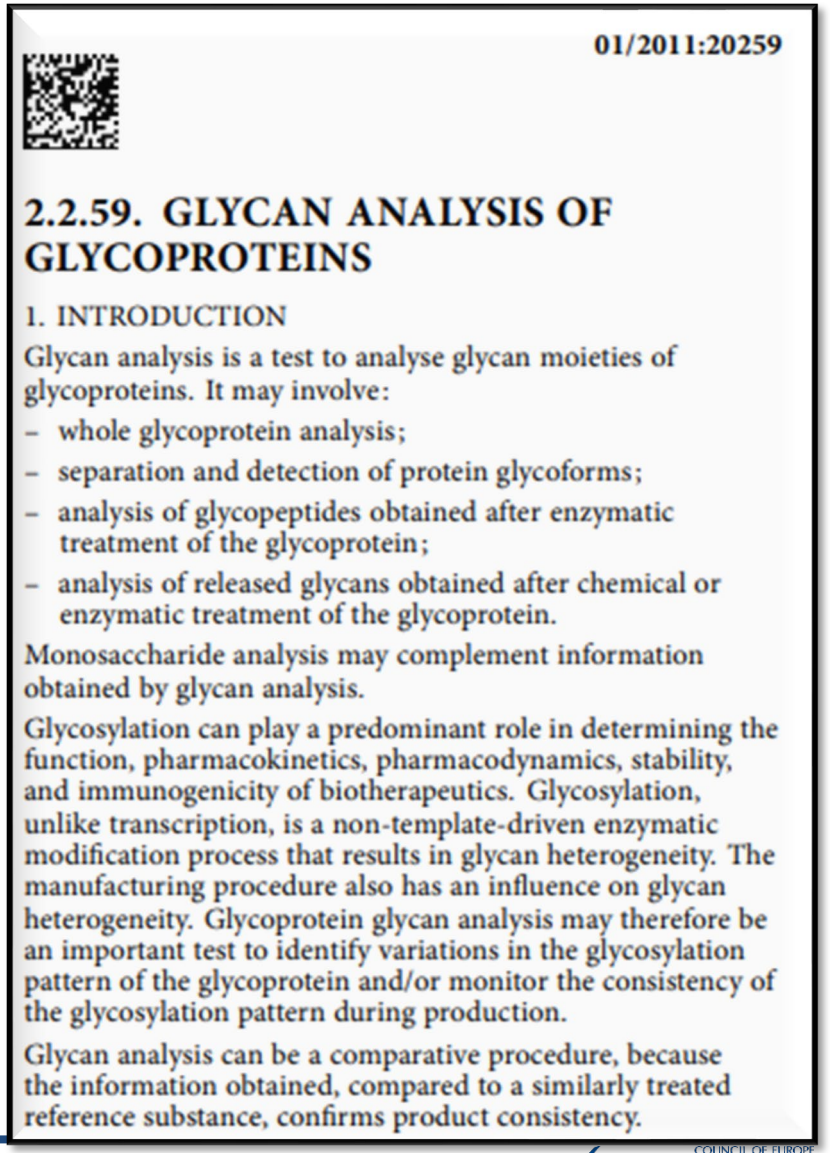


.....

Glycan Analysis of Glycoproteins (2.2.59) (1)

- ✓ Different approaches used for glycoprotein glycan analysis:
  - analysis of intact glycoprotein
  - analysis of glycopeptides
  - analysis of released glycans
  - monosaccharides analysis
- ✓ Requirements for application and validation of glycan analysis procedures
- ✓ Framework for selection of appropriate procedures
- ✓ Guidance on reference standards: 1) SST (e.g. fetuin, IgG); 2) for compliance testing (substance-specific)
- ✓ Links to other relevant general chapters, *e.g.* CE (2.2.47; MS (2.2.43); SEC (2.2.30); IEX (2.2.46); IEF (2.2.54)
- ✓ Points to consider in analytical procedure development
- ✓ Glycan analysis is not a single general method, but involves the application of specific procedures and development of specific glycan maps for each unique glycoprotein.

⇨ Specific procedures are therefore indicated in relevant specific monographs.



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2.2.59. GLYCAN ANALYSIS OF GLYCOPROTEINS

1. INTRODUCTION

Glycan analysis is a test to analyse glycan moieties of glycoproteins. It may involve:

- whole glycoprotein analysis;
- separation and detection of protein glycoforms;
- analysis of glycopeptides obtained after enzymatic treatment of the glycoprotein;
- analysis of released glycans obtained after chemical or enzymatic treatment of the glycoprotein.

Monosaccharide analysis may complement information obtained by glycan analysis.

Glycosylation can play a predominant role in determining the function, pharmacokinetics, pharmacodynamics, stability, and immunogenicity of biotherapeutics. Glycosylation, unlike transcription, is a non-template-driven enzymatic modification process that results in glycan heterogeneity. The manufacturing procedure also has an influence on glycan heterogeneity. Glycoprotein glycan analysis may therefore be an important test to identify variations in the glycosylation pattern of the glycoprotein and/or monitor the consistency of the glycosylation pattern during production.

Glycan analysis can be a comparative procedure, because the information obtained, compared to a similarly treated reference substance, confirms product consistency.

Glycan Analysis of Glycoproteins (2.2.59) (4)

The following monographs include reference to chapter 2.2.59:

> *"Use a suitable procedure developed according to general chapter 2.2.59. Glycan analysis of glycoproteins, section 2-3."*

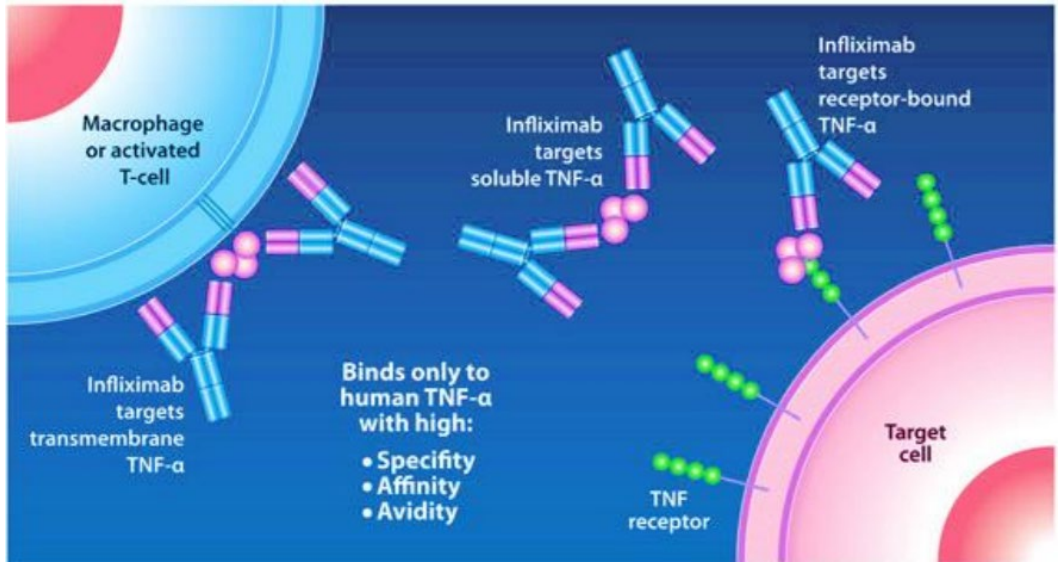
- Alteplase for injection (1170)
- Erythropoietin conc. solution (1316)
- Etanercept (2895)
- Follitropin (2285)
- Follitropin concentrated solution (2286)
- Human coagulation factor IX (rDNA) concentrated solution (2522)
- Human coagulation factor VIIa (rDNA) concentrated solution (2534)
- Infliximab concentrated solution (2928)

chapter 2.2.59 becomes mandatory

Standardisation of TNF-alpha Bioassays

- **Rapidly growing number** of TNF-alpha antagonists on the market
- **Increased variety of approaches** to bioassay selection for assessing and comparing potencies
- Questions raised concerning the **appropriate choice of potency assays** for particular products and how they should be designed, conducted, analysed and applied

Target Antigen	MOA
TNF-alpha	prevents TNF-alpha receptor activation by binding to TNF-alpha, thereby neutralising the biological activity of TNF-alpha



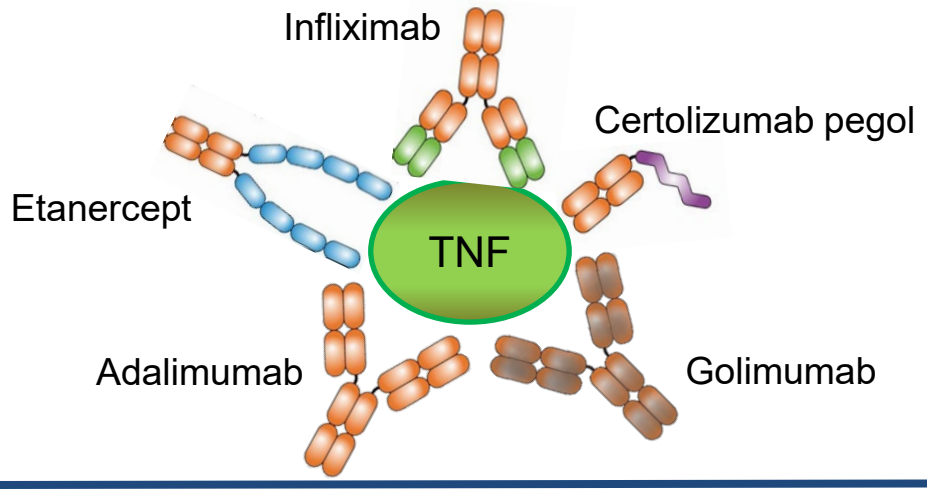
Biological activity evaluated in **cell-based potency assays** using different approaches for **TNF-alpha neutralisation**

Standardisation of TNF-alpha Bioassays

Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)

- analytical tools and practical guidance to further build on and support testing
- general methodologies for potency determination, widely applicable to the class of **TNF-alpha antagonists**

Bioassay "Horizontal Standard"



➢ Choice of assays and scope of validation/verification

TNF-alpha antagonist	U937 apoptosis assay - Procedure A -	WEHI-164 cytotoxicity assay - Procedure B -	NF-κB-inducible reporter gene assay - Procedure C -	L929 cytotoxicity assay - Procedure D -
Etanercept*	●	◗	◗	◗
Infliximab*	◗	●	◗	◗
Certolizumab pegol	◗	◗	●	◗
Adalimumab**	◗	◗	◗	●
Golimumab**	○	◗	○	○

● signifies that procedure has been validated
◗ signifies that suitability has been demonstrated during verification experiments
○ signifies that suitability has not been evaluated
* Ph. Eur. monograph
** Draft monograph under elaboration

TNF-alpha Bioassay Horizontal Standard

Cell-based assay for potency determination of TNF-alpha antagonists (2.7.26)*

- **NEW type of general chapter** with experimentally verified cell-based assays
- TNF-alpha neutralisation assays (procedures A, B, C and D):
  - ➔ different cell lines/readouts
  - ➔ validated for specific TNF-alpha antagonists
  - ➔ suitability (specificity and precision) demonstrated for each TNF-alpha antagonist, during verification experiments
  - ➔ assay applied to substances outside the scope of the initial validation or not covered in an individual monograph for a TNF-alpha antagonist requires validation
- Diversifies the choice of bioassays and facilitates migration to different assays
- Use of other assays that are acceptable to the competent authority not excluded

- Cell preparation
- TNF-alpha working solutions preparation
- Test solution preparation
- Reference solution preparation (product-specific: BRP or IHRS)
- Assay execution
- Dose-response curve construction
- Calculation of reportable result

*Ph. Eur. Supplement 11.1

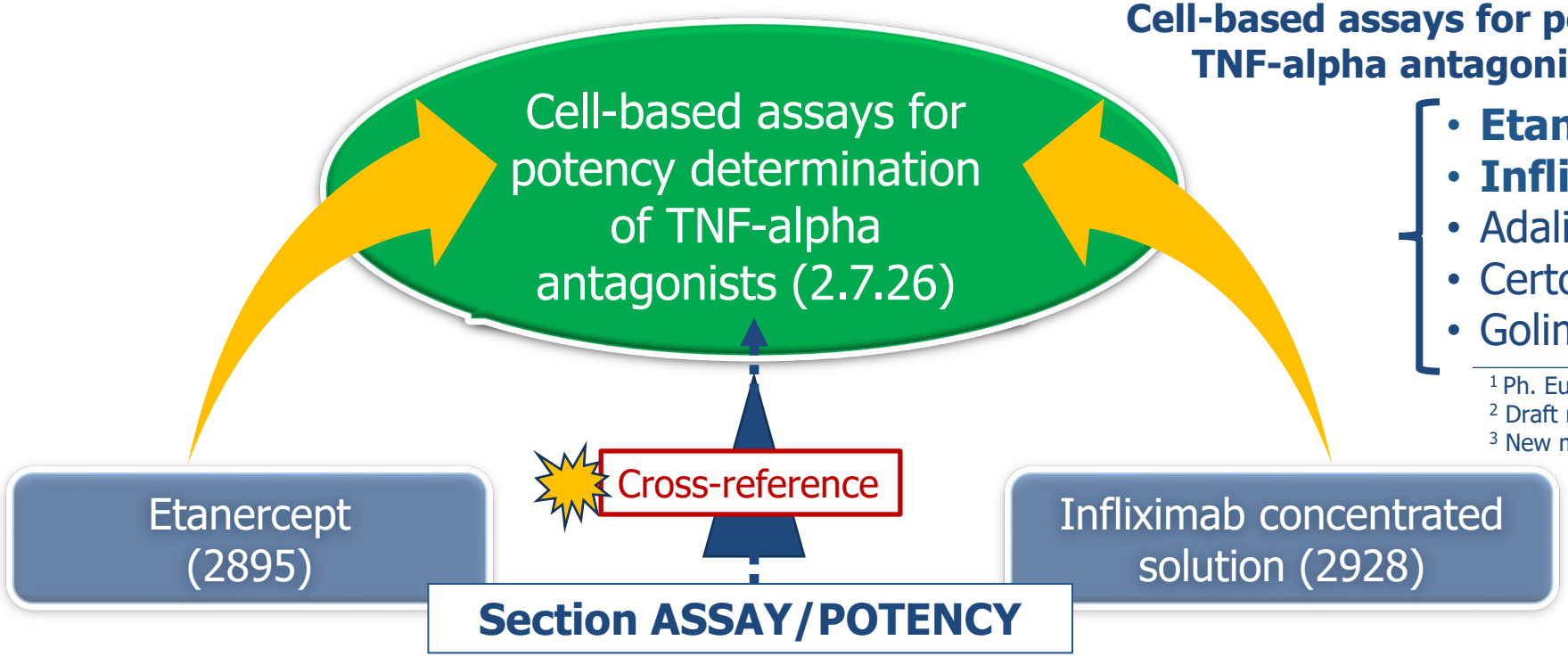
Analytical procedure control strategy

- ✓ system suitability test: quality of RS and control curves, proper functioning of the system (max to min ratio between controls)
- ✓ sample suitability assessment: compare performance of the sample to the performance of the RS (similarity/parallelism)
- ✓ **procedure-independent performance controls and one-size-fits all criteria**

Sources of variability identified and potential mitigation strategies described:

- ✓ adjustment of assay conditions to satisfy the system suitability criteria without fundamentally modifying the procedures

Link between Chapter and Individual Monographs



Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26) – scope:

- **Etanercept**[1]
- **Infliximab**[1]
- Adalimumab[2]
- Certolizumab pegol
- Golimumab[3]

[1] Ph. Eur. monograph
[2] Draft monograph under elaboration
[3] New monograph (to be published in Ph. Eur. 11.6)

- **Link created with monographs on TNF-alpha antagonists**
  - diversifies the choice of suitable bioassays for potency determination
  - reinforces and maintains the flexibility already built into the monographs and the use of Ph. Eur. reference standards

Link between Chapter and Individual Monographs

Potency. The potency of etanercept is determined by comparison of dilutions of the test preparation with the dilutions of *etanercept BRP* using a suitable cell-based assay based on the inhibitory action of etanercept on the biological activity of TNF-α and a suitable readout for assessing this inhibitory effect.

The following procedure is given as an example.

U937 apoptosis assay (*2.7.26, Procedure A*). Carry out the assay as described with the following modifications.

Test solution. Dilute the preparation to be examined with assay medium to obtain a concentration of about 21 ng/mL. Use this solution to prepare 10 additional test sample dilutions (a dilution step of 1.4 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

Reference solution. Reconstitute the contents of 1 vial of *etanercept BRP* with sterilised *water for injections R* to obtain a concentration of 10 000 IU/mL. Further dilute with assay medium to obtain a concentration of 42 IU/mL. Use this solution to prepare 10 additional reference sample dilutions on a dilution plate to generate the standard curve (a dilution step of 1.4 has been found suitable). Analyse 2 independent dilutions per plate.

Result: the estimated potency is not less than 80 per cent and not more than 140 per cent relative to the reference solution. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

In addition, the following procedures have been found suitable:

WEHI-164 cytotoxicity assay (*2.7.26, Procedure B*). Carry out the assay as described with the following modifications.

Test solution. Dilute the preparation to be examined with assay medium to obtain a concentration of about 96 ng/mL. Analyse 2 independent dilutions per plate.

Reference solution. Reconstitute the contents of 1 vial of *etanercept BRP* with sterilised *water for injections R* to obtain a concentration of 10 000 IU/mL. Further dilute with assay medium to obtain a concentration of 192 IU/mL. Analyse 2 independent dilutions per plate.

Plate preparation. Add 300 µL of the test or reference solutions (column 2, rows A-H). Further prepare a series of 1.5-fold dilutions (columns 3-12, rows A-H), by removing 200 µL from column 2 and transferring to the adjacent well in column 3, repeating for subsequent wells.

NF-κB-inducible reporter gene assay (*2.7.26, Procedure C*). Carry out the assay as described with the following modifications.

Test solution. Dilute the preparation to be examined with assay medium to obtain a concentration of about 1000 ng/mL. Use this solution to prepare 11 test sample dilutions in the range 1.0-200.0 ng/mL (a dilution step of 1.7 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

Reference solution. Reconstitute the contents of 1 vial of *etanercept BRP* with sterilised *water for injections R* to obtain a concentration of 10 000 IU/mL. Further dilute with assay medium to obtain a concentration of 400 IU/mL. Use this solution to prepare 10 additional reference sample dilutions (a dilution step of 1.7 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

L929 cytotoxicity assay (*2.7.26, Procedure D*). Carry out the assay as described with the following modifications.

Test solution. Dilute the preparation to be examined with assay medium to obtain a concentration of 45 ng/mL. Use this solution to prepare 11 test sample dilutions, starting from 10 ng/mL (a dilution step of 1.7 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

Reference solution. Reconstitute the contents of 1 vial of *etanercept BRP* with sterilised *water for injections R* to obtain a concentration of 10 000 IU/mL. Further dilute with assay medium to obtain a concentration of 20 IU/mL. Use this solution to prepare 10 additional reference sample dilutions (a dilution step of 1.7 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

Etanercept (2895)

Potency. The potency of infliximab is determined by comparison of dilutions of the test preparation with dilutions of *infliximab BRP* using a suitable cell-based assay based on the inhibitory action of infliximab on the biological activity of TNF-α with a suitable readout for assessing this inhibitory effect.

The following procedure is given as an example.

WEHI-164 cytotoxicity assay (*2.7.26, Procedure B*). Carry out the assay as described with the following modifications.

Reference solution. Reconstitute the contents of 1 vial of *infliximab BRP* with sterilised *water for injections R* to obtain a concentration of 500 IU/mL. Further dilute with assay medium to obtain a concentration of 6.4 IU/mL. Analyse 2 independent dilutions per plate.

Result: the estimated potency is not less than 80 per cent and not more than 120 per cent relative to the reference solution. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

In addition, the following procedures have been found suitable:

U937 apoptosis assay (*2.7.26, Procedure A*). Carry out the assay as described with the following modifications.

Reference solution. Reconstitute the contents of 1 vial of *infliximab BRP* with sterilised *water for injections R* to obtain a concentration of 500 IU/mL. Further dilute with assay medium to obtain a concentration of 12.5 IU/mL. Use this solution to prepare 10 additional reference sample dilutions on a dilution plate to generate the standard curve (a dilution step of 2 has been found suitable). Analyse 2 independent dilutions per plate.

NF-κB-inducible reporter gene assay (*2.7.26, Procedure C*). Carry out the assay as described with the following modifications.

Reference solution. Reconstitute the contents of 1 vial of *infliximab BRP* with sterilised *water for injections R* to obtain a concentration of 500 IU/mL. Further dilute with assay medium to obtain a concentration of 8 IU/mL. Use this solution to prepare 10 additional reference sample dilutions (a dilution step of 1.7 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

L929 cytotoxicity assay (*2.7.26, Procedure D*). Carry out the assay as described with the following modifications.

Reference solution. Reconstitute the contents of 1 vial of *infliximab BRP* with sterilised *water for injections R* to obtain a concentration of 500 IU/mL. Further dilute with assay medium to obtain a concentration of 1.0 IU/mL. Use this solution to prepare 10 additional reference sample dilutions (a dilution step of 1.7 has been found suitable) on a dilution plate. Analyse 2 independent dilutions per plate.

Infliximab concentrated solution (2928)

Acceptance criteria (relative potency by *example procedure*)

- **estimated potency** relative to RS
- **confidence limits (P = 0.95)**

Suitable TNF-alpha neutralisation assay – calibration with:

Etanercept BRP	Infliximab BRP
The following procedure is given as an example	
U937 apoptosis assay (*2.7.26, Procedure A*)	WEHI-164 cytotoxicity assay (*2.7.26, Procedure B*)
In addition, the following procedures have been found suitable	
2.7.26, Procedures B, C, D	2.7.26, Procedures A, C and D

"suitable", "example procedure" defined in Ph. Eur. General Notices

SLIDO

Potency of etanercept X is determined using an in-house U937 apoptosis assay with a different setup compared with the example procedure given in the Etanercept (2895) monograph. Is this product compliant with the Ph. Eur.?

- Yes, the monograph allows for flexibility as regards the assay format.
- Yes, with the note that the in-house reference standard used is to be established by comparison with the Ph. Eur. Etanercept BRP, to which it is traceable.
- No, the user needs to determine the specific activity of the protein in line with the assay section of the monograph and the result has to fulfil the monographs acceptance criteria.
- Yes, if the in-house potency assay is validated and demonstrated comparable to the pharmacopoeial procedure.

Correct answer in green!

Strategy for Ph. Eur. Quality Standards for *Multi-source* Monoclonal Antibodies: Monographs and Horizontal Standards

Ph. Eur. Standards for Multi-source Mabs: Approaches

- Target **product classes** and specific drug substance(s) (*built-in flexibility*)
- Develop **general methods of analysis** to support analytical testing: broad applicability, performance characteristics

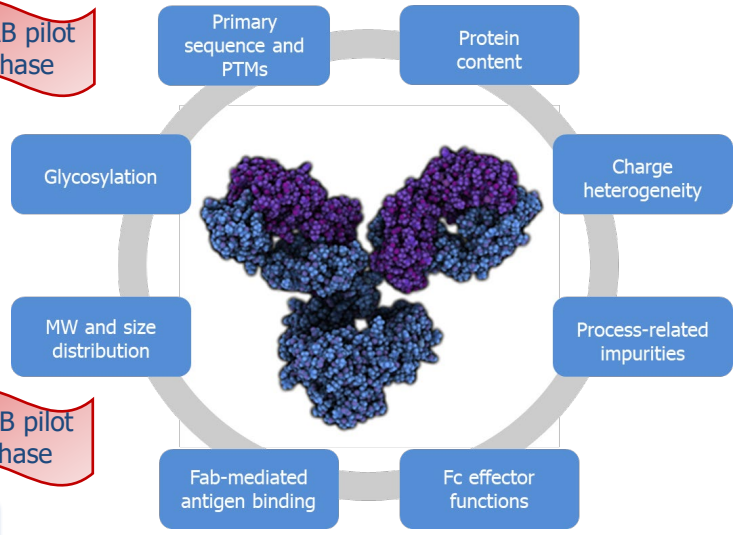
- Explore **flexible concepts** and **new types of standardisation** ("horizontal standards"):
  - Focus on key quality attributes and associated testing strategies
  - Establish suitable common expectations and general methodologies with broad applicability
  - Contribute to standardisation of therapeutic monoclonal antibodies through rationalisation of methodologies and common functionalities
  - Help guide analytical procedure development, enabling flexibility for the adoption of newer analytical technologies throughout the product lifecycle and the use of alternative methods

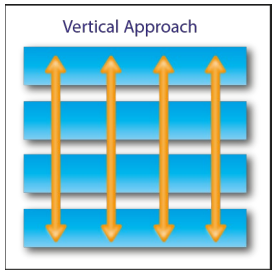
MAbs: Approaches to Public Standard-Setting

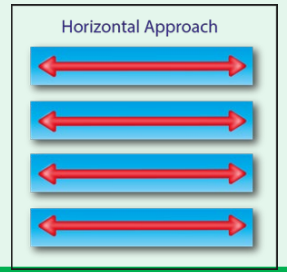
- Ustekinumab (3165)[1]
- Adalimumab (3147)[2] — MAB pilot phase
- Golimumab concentrated solution (3103)[3]
- Infliximab concentrated solution (2928)[4] — MAB pilot phase
- Etanercept (2895)

Definition
Production
Identification
Tests
Assay/Content
Assay/Potency

Monographs

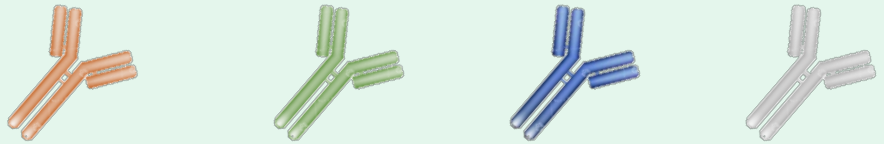






Horizontal standards (general chapters)

MAB pilot phase



Product-class standard

Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)

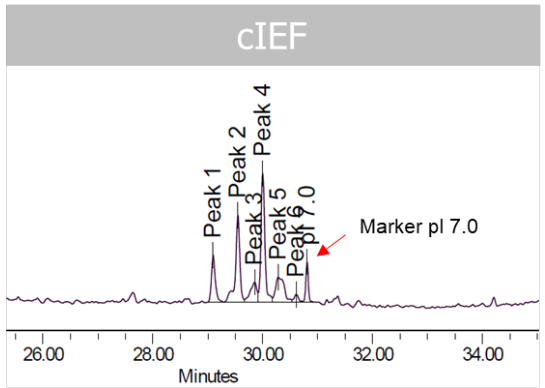
Performance-based standards

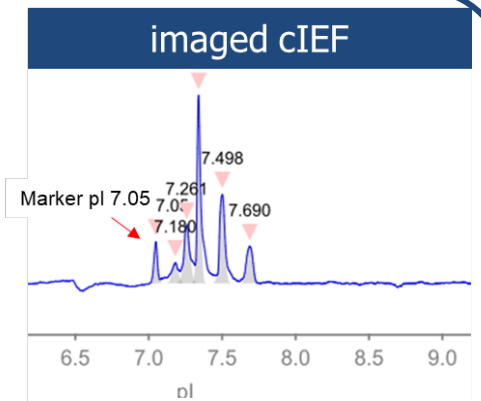
- Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies (2.5.43)*
- Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies (2.5.44)*

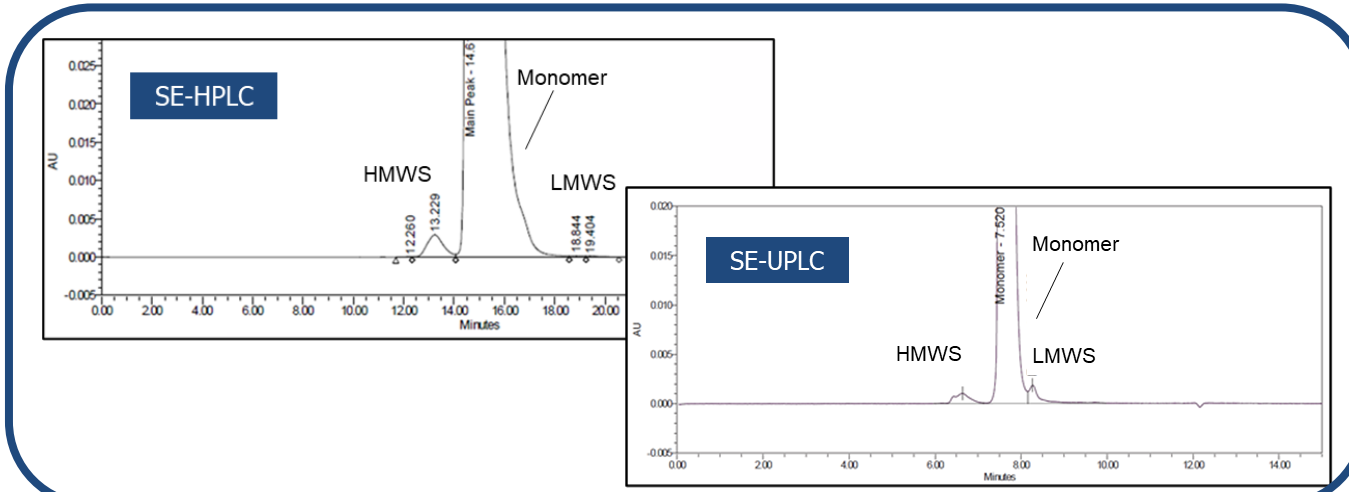
[1]under elaboration; [2]under elaboration as part of MAB pilot phase;
[3]to be published in Ph. Eur. Suppl. 11.6 (07/2024); [4]Ph. Eur. monograph elaborated as part of MAB pilot phase

Performance-based Standards: Key Aspects

- Based on validated analytical procedures (mAb-specific), extended to a wide range of mAbs
- Evaluation of selected analytical procedures through **collaborative studies involving multiple laboratories**, with the aim to:
  - verify their applicability as suitable generic/multi-product procedures for mAb analysis
- Knowledge/data gathered on:
  - analytical procedure performance characteristics and associated criteria
  - system suitability, system performance, assay acceptance criteria
  - requirements for peak resolution and guidance on peak integration approaches
  - identification of appropriate controls and reference materials







Horizontal Standard Development Beyond Product Class

Pharmeuropa 35.4 (Oct. – Dec. 2023)

- *2.5.44 Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies:*
  - cIEF and imaged cIEF procedures for analysis of charge heterogeneity of mAbs, to monitor identity, quality, production consistency
  - based on data generated in multi-laboratory study
  - system performance, system suitability and assay acceptance criteria; use of reference standards
  - guidance on aspects to consider for product-specific application (development and validation)

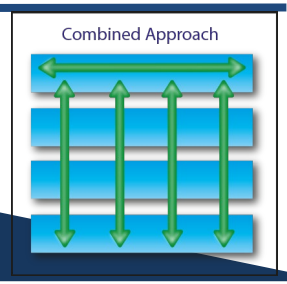
- *2.5.43 Size exclusion chromatography for recombinant therapeutic monoclonal antibodies:*
  - widely used methodology for determination of size variants (monomer, HMWS); quantitation of LMWS can be highly variable depending on the mAb analysed
  - multi-product SE-HPLC and SE-UPLC procedures, given as examples
  - suitability of selected SEC procedures demonstrated by collaborative study

"Performance-based standards"

➡ well-defined analytical procedures and **tools to control analytical procedure performance** (including reference materials)
➡ facilitate evaluation of key quality attributes of mAbs (charge heterogeneity, size variants)

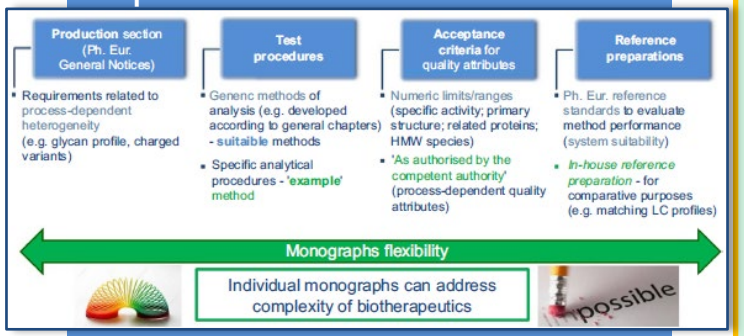
Ph. Eur. Standards for mAbs: Summary

Ph. Eur. Standards for Therapeutic Monoclonal Antibodies: Development Approaches



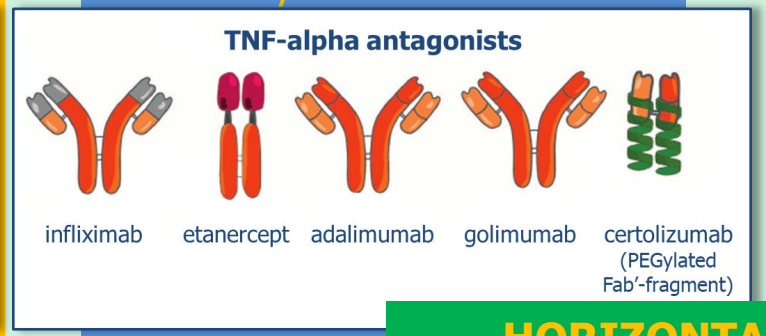
MONOGRAPHS*

- built-in *flexibility*
- *examples* of suitable procedures



PRODUCT CLASS - BASED STANDARDS

- product classes/sub-classes quality attributes
- *TNF-alpha neutralisation*



PERFORMANCE-BASED STANDARDS

- *platform* methodologies
- performance characteristics
- reference standards

HORIZONTAL STANDARDS

PRODUCT KNOWLEDGE, CASE STUDIES, COLLABORATIVE TESTING

* Buda M., Kolaj-Robin O., Charton E. *Biotherapeutic Products in the European Pharmacopoeia: Have all Challenges Been Tackled?* Generics and Biosimilars Initiative Journal. 2022;11(1)
Buda M. *Development of Ph. Eur. standards for therapeutic monoclonal antibodies: infliximab case study.* Generics and Biosimilars Initiative Journal. 2022;11(3)

Survey on Strategy for Quality Standards for mAbs

- **MAB pilot phase**: aim to explore feasibility of establishing quality standards for mAbs based on the specifications of more than one marketed product ("multi-source mAbs"), using a twofold approach:
  - develop general chapters on analytical procedures applicable to a wide range or to classes of mAbs ("**horizontal standards**")
  - elaborate individual monographs for multi-source mAbs.
- **Survey aims to gather feedback** on the approach taken in the "MAB pilot phase", based on concrete examples.

Click here to participate in the survey

- Deadline for participation: 31 March 2024 -

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

edqm

European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE

CONSEIL DE L'EUROPE

Module 1: General concepts. Biotherapeutics and ATMPs

30 JANUARY 2024 - 13:00-16:15 (CET, FRANCE)

2024 EDQM TRAINING PROGRAMME ON EUROPEAN PHARMACOPOEIA TEXTS RELATED TO BIOLOGICALS AND ON MICROBIOLOGY CHAPTERS

Ph. Eur. general concepts

Emmanuelle Charton, Mihaela Buda, EDQM, Council of Europe

Biotherapeutics

Mihaela Buda, EDQM, Council of Europe

Advanced therapy medicinal products (ATMPs)

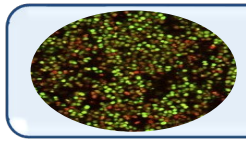
Olga Kolaj-Robin, EDQM, Council of Europe

Cell and gene therapy – Ph. Eur. portfolio

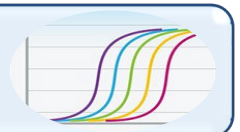
General overarching texts

- 5.14 Gene transfer medicinal products for human use

- 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products



General methods: numeration & viability



- 2.7.23 Numeration of CD34+/CD45+ cells in haematopoietic products
- 2.7.24 Flow cytometry
- 2.7.28 Colony-forming cell assay for human haematopoietic progenitor cells
- 2.7.29 Nucleated cell count and viability
- 2.6.35 Quantification and characterisation of host-cell DNA

General chapters: Microbiology aspects & viral safety

- 2.6.1 Sterility
- 5.1.6 Alternative methods for control of microbiological quality
- 2.6.27 Microbiological examination of cell-based preparations
- 2.6.39 Microbiological examination of human tissues
- 2.6.14 Bacterial endotoxins - 2.6.30 MAT - 2.6.32 rFC
- 2.6.7 Mycoplasmas
- 5.1.7 Viral safety
- 5.2.8 TSE

Monographs

- Bovine serum (2262)
- Human haematopoietic stem cells (2323)

Non exhaustive list

Cell and gene therapy – Ph. Eur. portfolio

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- 5.14 Gene transfer medicinal products for human use

under revision
Recently published/revised

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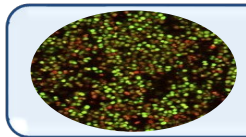

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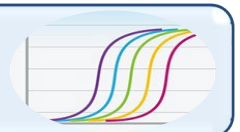

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under revision
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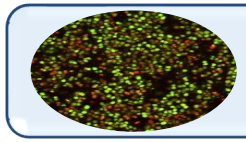

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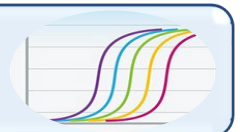

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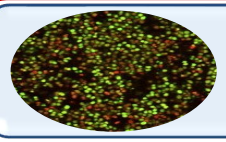

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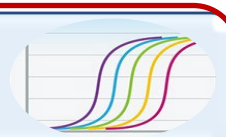

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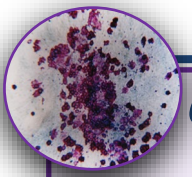
Non exhaustive list

General methods – revised texts

REVISION



Colony-forming cell assay for human haematopoietic progenitor cells (2.7.28)

1. *Introduction*
2. *CFC specificity*
3. *QA for CFC assay*
4. *CFC assay*
   - *4.1 Materials*
   - *4.2 Cell culture*
   - *4.3 Plating*
   - *4.4 Enumeration and identification of the colonies*
   - *4.5 Expression of the results*
5. *Analytical procedure validation*
6. *Glossary*

- Clarification of definition of **colony-forming cells** (CFCs) and their functional capacity

Enhanced standardisation

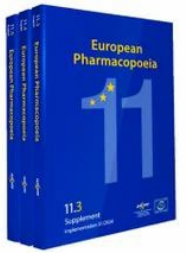
- Importance to **control and document source of materials**
- Recommend using materials with **low level of bacterial endotoxins**
- Recommendation of **serum-free medium** and **recombinant growth factors**
- Address the **cell processing prior to CFC assay** (e.g. depletion of erythrocytes for freshly collected cells)
- **Replicate dishes seeded** for the examination of a suspension of single cells
- Standardisation using the **number of CD34/CD45+ cells seeded by plate**
- **Commercially available media** encouraged

Inclusion of fully and semi-automated systems

More detailed recommendations on analytical validation

Supplement 11.3

Implementation: 1 January 2024



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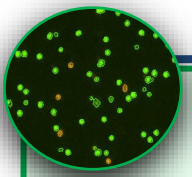
General methods – revised texts



Nucleated cell count and viability (2.7.29)

1. General considerations

2. Technical considerations

- 2.1. Sample preparation and test conditions
- 2.2. Dye-exclusion methods

3. Manual cell counting and viability

- 3.1. Cell count
- 3.2. Viability analysis

4. Automated cell counting and viability

- 4.1. Cell count
- 4.2. Viability analysis
- 4.3. Methods
  - Flow cytometry
  - Image cytometry

5. Procedure validation

- Prerequisites
- Suitability of sample material
- Recommended experimental design
- Recommendation on validation parameters

Improvement of the standardisation

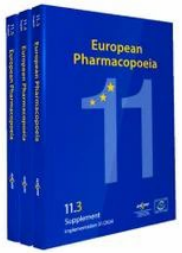
Table summarising information on commonly used dyes

Addition of image cytometry

Table summarising main characteristics of flow cytometry and image cytometry

Recommendations on analytical validation

Supplement 11.3



Implementation: 1 January 2024

Can I use *Nucleated cell count and viability (2.7.29)* in the context of cells used in a bioassay?

- ❑ Yes
- ❑ No
- ❑ I don't know

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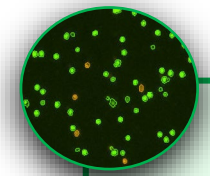

Nucleated cell count and viability (2.7.29)

- **Chapter scope: nucleated cell count and viability for cell suspension**
- **Elaborated with cellular products in mind but it may be applicable in other cases such as cells used in bioassay**
- **Considerations on method validation provided in the chapter**

☑ Yes

Cell and gene therapy – Ph. Eur. portfolio

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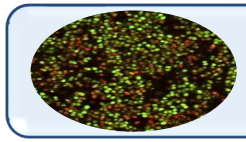

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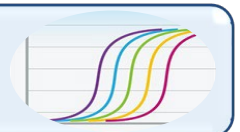

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Monographs

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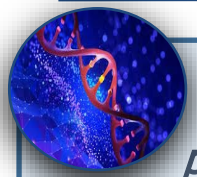
- Human haematopoietic stem cells (2323)

Non exhaustive list

General overarching texts – GTP texts



Gene transfer medicinal products for human use (5.14)

- Definition, Production
  - *Recombinant vectors*
  - *Genetically modified cells*
- Plasmid vectors for human use
- Bacterial cells used for the manufacture of plasmid vectors for human use
- Adenovirus vectors for human use
- Poxvirus for human use

- Adeno-associated-virus vectors for human use
- Retroviridae-derived vectors for human use

Adopted 2005
Ph. Eur. 5.6
(Jan 2007)

Adopted 2008
Ph. Eur. 6.6
(Jan 2010)

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

- Scope
- Risk assessment
- General requirements
- Sera and serum replacements
- Proteins produced by rDNA technology
- Proteins extracted from biological material
- Vectors

Adopted 2015
Ph. Eur. 9.0
(Jan 2017)

Which text do I have to follow for my intravenous AAV vector-based GTMP?

- ❑ Gene transfer medicinal products for human use (5.14)
- ❑ Raw materials for cell-based and gene therapy medicinal products (5.2.12)
- ❑ Products of recombinant DNA technology (0784)
- ❑ Pharmaceutical preparations (2619)
- ❑ Live biotherapeutic products for human use (3053)
- ❑ Substances for pharmaceutical use (2034)
- ❑ Parenteral preparations (0520)
- ❑ Products with risks of transmitting agents of animal spongiform encephalopathies (1483)
- ❑ I don’t know

Which text do I have to follow for my AAV intravenous vector-based GTMP?

There is currently no <u>monograph</u> specific for AAV vectors in the Ph. Eur.

- ❑ Gene transfer medicinal products for human use (5.14)
- ❑ Raw materials for cell-based and gene therapy medicinal products (5.2.12)
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- ☑ Parenteral preparations (0520)
- ☑ Products with risks of transmitting agents of animal spongiform encephalopathies (1483)
- ❑ I don't know

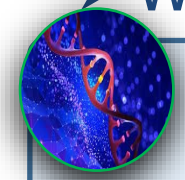
General monographs apply

- 0784 – not applicable to recombinant organisms intended to be used directly in man or animals e.g. recombinant vectors or vaccines
- 3053 – covers medicinal products containing live microorganisms (bacteria or yeasts) for human use applied orally or vaginally; FMT and GTP excluded

(5.14), (5.2.12) not legally binding but widely applied

General overarching texts – GTP texts

- General chapters → not legally binding **but** reflecting the consensus of Ph. Eur. member states
- The provisions of the chapter **do not exclude** the use of different production and control methods.
- Widely recognised and used



Gene transfer medicinal products for human use (5.14)

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Guidelines relevant for advanced therapy medicinal products

Human | Advanced therapies | Regulatory and procedural guidance | Research and development

European Pharmacopoeia

The following monograph from the European Pharmacopoeia (Ph. Eur) should be considered, where relevant:

- Ph.Eur. monograph 5.14 **Gene transfer medicinal products** for human use (01/2010:51400)
- General chapter 5.2.12 **Raw materials for the production of cell-based and gene therapy medicinal products**

22 March 2018
EMA/CAT/80183/2014
Committee for Advanced Therapies (CAT)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products

12 November 2020
EMA/CAT/GTWP/671639/2008 Rev. 1 - corr
Committee for Advanced Therapies (CAT)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells

EUROPEAN COMMISSION

EudraLex
The Rules Governing Medicinal Products in the European Union
Volume 4
Good Manufacturing Practice

Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products

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5.2.12 Raw materials for cell-based and gene therapy medicinal products

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

- To identify the critical quality attributes of raw materials
- To harmonise variable practices
- To encourage raw materials manufacturers to:
  - provide consistent, predefined quality
  - record and share information on the origin and quality of the raw material
- To help users to manage batch-to-batch variations and changes in raw materials

- Address the quality of raw materials at **early stage of development** to avoid extra work
- **Clarifies responsibilities**
  - **manufacturer of a raw material to qualify** (prove to be suitable for the intended use) the raw material in accordance with the requirements given in the general chapter
  - **user of a raw material to ensure it is of suitable quality** for the specific use (ultimate responsibility)

Is serum- and xeno-component free medium in scope of 5.2.12?

- ❑ Yes
- ❑ No
- ❑ I don’t know
- ❑ It depends

Is serum- and xeno-component free medium in scope of 5.2.12?

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

No, if medium fully synthetic

Yes, partially, if it contains additives such as e.g. human serum albumin

Applies to:

- sera and serum replacement
- proteins produced by recombinant DNA technology
- proteins extracted from biological materials
- vectors

Not in the scope:

- chemically synthesised raw materials: e.g. basal media (purely composed of chemicals)
- synthetic peptides or polynucleotides
- medical devices and plastics

☑ It depends

5.2.12 Raw materials for cell-based and gene therapy medicinal products

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

- Scope
- **Risk assessment**
- General requirements
- Sera and serum replacements
- Proteins produced by rDNA technology
- Proteins extracted from biological material
- Vectors

Risk assessment

- Evaluation of the RM impact on the quality, safety and efficacy of cell-based/gene therapy medicinal product to be performed by the user
- Risk factors evaluated in relation to the clinical benefit/risk

To consider:

- biological origin
- traceability of the raw material
- production steps
- ability of the drug product manufacturing process to control or remove the raw material from medicinal product

5.2.12 Raw materials for cell-based and gene therapy medicinal products

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

- Scope
- Risk assessment
- **General requirements**
- Sera and serum replacements
- Proteins produced by rDNA technology
- Proteins extracted from biological material
- Vectors

General Requirements

- **Minimise the use of raw materials of human or animal origin**
- **Traceability required**
  - Human origin materials – each donation to be followed from the donation to the raw material and to the final product, and vice versa
  - Animal origin materials – if origin not fully traceable, information of their geographic location at sourcing time
  - Vectors or rDNA proteins – traceability to MCB/virus seed lot
- **Minimisation of the risk of transmitting adventitious agents**
  - Human origin materials – carefully evaluated donors adequately tested for infectious transmissible agents (compliance with appropriate EU and/or national legislation)
  - Animal origin materials – specific health requirements, fit for human consumption and reared under controlled conditions
  - Viral risk assessment (extent dependent on the original risk assessment) according to *Viral safety (5.1.7)*
  - Transmissible spongiform encephalopathies – risk assessment and minimisation of the risk according to TSE chapter *(5.2.8)*
  - Special attention to pooling (limitation of pooled donations)
  - Sufficient methods/production process optimised for inactivation/removal of adventitious agents)

5.2.12 Raw materials for cell-based and gene therapy medicinal products

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

- Scope
- Risk assessment
- **General requirements**
- Sera and serum replacements
- Proteins produced by rDNA technology
- Proteins extracted from biological material
- Vectors

General Requirements: cont.

Must meet pre-defined quality requirements for identity, purity and biological activity

- Identification – specific for the RM, addresses molecular structure/composition or other physico-chemical, biological or immunochemical properties
  - Appearance (2.2.1 & 2.2.2)
  - Solubility
  - Osmolality (2.2.35)
  - pH (2.2.3)
  - Elemental impurities
  - Total protein (2.5.33)
  - Viral contamination
  - BET (2.6.14)
  - Mycoplasmas (2.6.7)
  - Water (5.2.12)
  - Related substances
- Microbiological control – Sterility (2.6.1) or microbial contamination (2.6.12)
- Stabiliser (including antibiotics) – presence justified, impact assessed
- Content
- Biological activity (where relevant)

- Use of reference material or representative reference batch; Ph. Eur. or WHO IS recommended where available

5.2.12 Raw materials for cell-based and gene therapy medicinal products

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

- Scope
- Risk assessment
- General requirements

- **Sera and serum replacements** (incl. blood and other cellular components, platelet lysates, conditioned media)
- **Proteins produced by rDNA technology** (incl. Growth factors, cytokines, hormones, enzymes and mAbs)
- **Proteins extracted from biological material** (incl. enzymes (e.g. trypsin), polyclonal Abs, other proteins (e.g. albumin), peptides)
- **Vectors** (incl. DNA vectors (e.g. plasmids, transposon vectors), viral vectors and bacteria (e.g. modified *Lactococcus* species)

+ Specific (more detailed) requirements

➢ Sera and serum replacements

- Special attention to verifying the batch consistency and performance
- Cell bank system preferred for conditioned media
- Additional tests for haemoglobin, cell-derived impurities, specific viral contaminants
- Reference to viral safety in *Bovine serum (2262)* and *Human plasma for fractionation (0853)* (for human serum) monographs

➢ rDNA proteins

- Well-characterised host-vector system, using MCB & WCB
- Particular attention to product-related impurities
- Additional tests for HCP, hcDNA, vector DNA

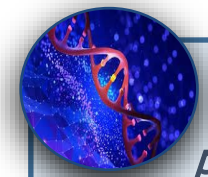
➢ Proteins extracted from biological material

- Reducing levels of process-related impurities such as blood components, tissue fragments or contaminating proteins
- Particular attention to product-related impurities (e.g. antibodies with undefined specificity, degradation and oxidation products, oligomers and aggregates)

➢ Vectors

- Principles of the chapter apply; reference to 5.14

5.14 Gene transfer medicinal products for human use



Gene transfer medicinal products for human use (5.14)

- Definition, Production
  - *Recombinant vectors*
  - *Genetically modified cells*
- Plasmid vectors for human use
- Bacterial cells used for the manufacture of plasmid vectors for human use
- Adenovirus vectors for human use
- Poxvirus for human use
- Adeno-associated-virus vectors for human use
- Retroviridae-derived vectors for human use

- Provides framework of requirements applicable to the production and control of the products
- Applicable for approved products
- Application to products used during clinical trials decided by the competent authority
- Alternative production and control methods acceptable to the competent authority not excluded

Ph. Eur. Gene Therapy Products texts: built-in flexibility

pDNA **Biological activity. Wherever possible,** biological activity is assessed (…). **It may be necessary** to supplement the bioassay with WB and ELISA assay to assess the integrity and quantity of the expressed product

AdV **Vector particle concentration.** It is performed by a **suitable technique** (**for example,** liquid chromatography, absorbance measurement or NAT (2.6.21)).

Gene transfer medicinal products for human use (5.14)

RV **AAV** **Expression of the genetic insert product.** It is determined **wherever possible,** following inoculation of cell cultures with the product at a predetermined multiplicity of infection, **by suitable** immunochemical (2.7.1) **or** biochemical assays **or** by flow cytometry (2.7.24). **Pox** **AdV**

AAV **AdV** **Replication-competent adenovirus/AAV concentration: within the limits approved** for the particular preparation.

EXAMPLES

The quality of the raw materials **may be considered according to the stage of development** of the cell-based or gene therapy medicinal product […].

Content. The content (e.g. protein content)/composition of the raw material is determined by an **appropriate qualified method.**

Stabiliser. Where applicable, it complies with the **limits defined for the particular raw material.**

- ✓ **Flexible wording**
- ✓ **Suitable methods**
- ✓ **No numerical acceptance criteria**

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)

Biological activity. **Where relevant,** the biological activity is determined by a **suitable assay. Where relevant** (e.g. for enzymes), the biological activity is expressed per milligram of total protein (specific activity).

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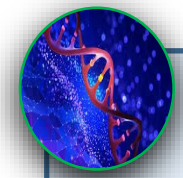
Is the section *Plasmid vectors for human use* applicable for plasmids used for preparation of vectors for subsequent genetic modification of cells?

- ❑ Yes
- ❑ No
- ❑ I don’t know

Is the section *Plasmid vectors for human use* applicable for plasmids used for preparation of vectors for subsequent genetic modification of cells?



Gene transfer medicinal products for human use (5.14)

Plasmid vectors for human use

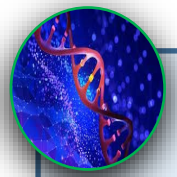
DEFINITION

(…) They are used to transfer genetic material into human somatic cells *in vivo* or to genetically modify autologous, allogeneic, xenogeneic or bacterial cells before administration to humans. (…)

Requirements for plasmids used for production of recombinant vectors outlined in individual sections.

☑ No

5.14 Gene transfer medicinal products for human use – AAV vectors



Gene transfer medicinal products for human use (5.14)

ADENO-ASSOCIATED-VIRUS VECTORS FOR HUMAN USE

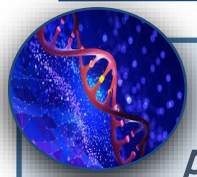
Definition
Production
- **Vector construction**
- **Vector production**
  - Packaging and producer cells
  - Plasmids
  - Viruses used for production
- **Production and harvest** (single harvest, control cells)
- **Purified harvest**
- **Final bulk**
- **Final lot** (ID, Tests, Assay, Labelling)

- Complete description (including ID, source, means of isolation, sequence, source and function of plasmid components)
- Production based on bacterial cell-bank system

BACTERIAL CELLS USED FOR THE MANUFACTURE OF PLASMID VECTORS FOR HUMAN USE

- Identification
- Genomic integrity
- Plasmid DNA (concentration)
- Residual host-cell DNA
- Bacterial endotoxins
- Sterility

GTP texts: ongoing work



Gene transfer medicinal products for human use (5.14)

- Definition, Production
  - *Recombinant vectors*
  - *Genetically modified cells*
- Plasmid vectors for human use
- Bacterial cells used for the ma
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- Poxvirus for human use

- Adeno-associated-virus vectors for human use
- Retroviridae-derived vectors for human use

Gene therapy medicinal products for human use (3186)

Definition
General requirements on:
- the Production of GTMPs
Recombinant vectors (including GMBCs)
enetically modified cells
tically modified autologous human cells
-associated-virus vectors for human use
ytic herpes simplex virus for human use

al information on gene therapy al products for human use (5.34)

nid vectors for human use
cterial cells used for the manufacture of plasmid vectors for human use
Genetically modified bacterial cells for human use
Adenovirus vectors for human use
oxvirus vectors for human use
etroviridae-derived vectors for human use

5.14 envisaged to be replaced by the new texts

References in 5.2.12 to be adjusted

Revised from 5.14
New sections

Ph. Eur. Gene Therapy Products texts

700 comments
Finalisation ongoing

- **Great interest raised in Pharmeuropa**
- **Finalisation ongoing**
- **Dedicated event envisaged upon finalisation**

Thank you for your attention

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